
From: Ikhlas Khan <ikhan@olemiss.edu>
Sent: Wednesday, August 02, 2017 2:29 PM
To: Geathers, LaQuia
Subject: Automatic reply: Revised budget

I am out of office till August 4th. Please contact Ms Jennifer Taylor jnnfrtyl@olemiss.edu , by email or call at 662 915 1090 if you need immediate attention.

From: Ikhlas Khan <ikhan@olemiss.edu>
Sent: Wednesday, September 02, 2015 8:56 AM
To: Pendleton, Kimberly
Cc: research@olemiss.edu; Welch, Cara; CFSAN Awards Mailbox; Geathers, LaQuia
Subject: Re: Notice of Grant Award (U01FD004246-05) Ikhlas A. Khan, PhD

Dear Kim
Thanks. Appreciate your help.
Ik

Sent from my iPhone

On Sep 2, 2015, at 8:32 PM, Pendleton, Kimberly <Kimberly.Pendleton@fda.hhs.gov> wrote:

Good Morning Dr. Khan,

Please find enclosed your FY2015 Notice of Grant Award. If you should have any questions, please don't hesitate to contact me.

Best,
Kim
Kimberly Pendleton Chew, FDA
CGMO/Branch Chief

<2689_001.pdf>

From: Calvey, Elizabeth M
Sent: Monday, June 02, 2014 12:31 PM
To: Geathers, LaQuia
Cc: Welch, Cara
Subject: FW: 1U01FD004246 Grant Progress Report
Attachments: 1U01FD004246 Grant Progress Report 060214.pdf

FYI

From: GRAY DALE [mailto:gdale@olemiss.edu]
Sent: Monday, June 02, 2014 9:37 AM
To: Hubbard, Vieda
Cc: Welch, Cara; Calvey, Elizabeth M; Ikhlas Khan; Scottie Casey; Anita Randle; AMAR GOPAL CHITTIBOYINA
Subject: 1U01FD004246 Grant Progress Report

Dear Ms. Hubbard,

Please see the attached grant progress report for 1U01FD004246. A hard copy of this report will be routed to you via FedEx (tracking number to follow). Can you please provide a full physical address for this shipment? Please let us know if you need further information regarding this report. Thank you.

Gray Dale

Project Coordinator

National Center for Natural Products Research
The University of Mississippi
School of Pharmacy
Technical Services Group
P.O. Box 1848
Thad Cochran Research Center 1014
University, MS 38677-1848
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"The time is always right to do what is right." --- Martin Luther King Jr.

Department of Health and Human Services
Public Health Services

Review Group

Type

Activity

Grant Number

1U01FD004246

Grant Progress Report

Total Project Period

From: 09/15/2011

Through: 08/31/2011

Requested Budget Period

From: 09/1/2014

Through: 08/31/2015

1. TITLE OF PROJECT

Science Based Authentication of Dietary Supplements

2a. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR

(Name and address, street, city, state, zip code)

Khan, Ikhlas A.
120 Faser Hall/NCNPR
School of Pharmacy
University of Mississippi
University, MS 38677

2b. E-MAIL ADDRESS

ikhan@olemiss.edu

2c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT

National Center for Natural Products Research

2d. MAJOR SUBDIVISION

School of Pharmacy

2e. Tel: 662-915-7821

Fax: 662-915-7989

3a. APPLICANT ORGANIZATION

(Name and address, street, city, state, zip code)

The University of Mississippi
Office of Research and Sponsored Programs
100 Barr Hall PO Box 907
University, MS 38677

3b. Tel: 662-915-7482

Fax: 662-915-7577

3c. DUNS: 067713560

4. ENTITY IDENTIFICATION NUMBER

1646001159A1

6. HUMAN SUBJECTS ☒ No ☐ Yes

6a. Research Exempt

☒ No ☐ Yes

If Exempt ("Yes" in 6a):

Exemption No.

If Not Exempt ("No" in 6a):

IRB approval date

5. NAME, TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL

Mickey McLaurin, Director of Sponsored Programs
Office of Research and Sponsored Programs
100 Barr Hall or PO Box 907, University MS 38677

Tel: 662-915-7482

Fax: 662-915-7577

E-MAIL: research@olemiss.edu

6b. Federal Wide Assurance No.

6c. NIH-Defined Phase III

Clinical Trial ☒ No ☐ Yes7. VERTEBRATE ANIMALS ☐ No ☒ Yes

7a. If "Yes," IACUC approval Date 06-20-12

7b. Animal Welfare Assurance No. A3356-01

10. PROJECT/PERFORMANCE SITE(S)

Organizational Name: University of Mississippi

DUNS: 067713560

8. COSTS REQUESTED FOR NEXT BUDGET PERIOD

8a. DIRECT \$1,798,535

8b. TOTAL \$2,500,000

Street 1: 120 Faser Hall/NCNPR

Street 2: School of Pharmacy

9. INVENTIONS AND PATENTS ☒ No ☐ Yes

If "Yes,"

☐ Previously Reported☐ Not Previously Reported

City: University

County: Lafayette

State: MS

Province:

Country: USA

Zip/Postal Code: 38677

Congressional Districts: MS-001

11. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Item 13)

Mickey McLaurin, Director of Sponsored Programs Administration

TEL: 662-915-7482

FAX: 662-915-7577

E-MAIL: research@olemiss.edu

12. Corrections to Page 1 Face Page

13. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN

11. (In ink)

DATE



5/30/14

Program Director/Principal Investigator (Last, First, Middle): Khan, Ikhlas, A.

			Salary Req.	Fringe	Total
Personnel					
PI	Ikhlas Khan	45%	(b)	(6)	
Co-PI	Larry Walker				
Principle Res.					
Scientist	Shabana Khan	23%			
Post Doc	Amira Wanas	50%			
Sr. Research					
Scientist	Bharthi Avula	70%			
Sr. Research					
Scientist	Yan Hong Wang	100%			
Res. Scientist	Natascha Techen	100%			
Res. Scientist	Zulfiqar Ali	25%			
Sr. Research					
Scientist	Amar Chittiboyina	50%			
Res. Scientist	Guoyi Ma	100%			
Res. Scientist	Ahmad Osman	100%			
Assoc. Res. Scientist	Jianping Zhou	53%			
Post Doc	Vijayasankar Raman	100%			
Post Doc	Mei Wang	70%			
Post Doc	Cristina Avonto	100%			
Post Doc	Vamshikrishna Manda	100%			
Post Doc	Zhihao Zhang	100%			
Post Doc	Min Hye Yang	100%			
Assoc. R&D Biologist	Helaina Craig	100%			
	Satyanarayanaraju				
Post Doc	Sagi	100%			
Post Doc	Pradeep Lasonkar	100%			
Post Doc	Iffat Parveen	100%			
R&D Botanist	Lal Jayaratna	100%			
R&D Data Analyst	Steven Hopper	100%			
Project Coordinator	Gray Dale	25%			
Program Coordinator	Jennifer Taylor	100%			
Hourly Wages		100%			
Graduate Students					
(4)		100%			
Total Salaries and FB					\$1,241,419
equipment					\$150,000
supplies					\$165,181
travel					\$40,000
contractual services					\$140,000
MOBOT					\$54,296
Subtotal					\$1,790,896
F&A 44%					\$709,104
Total Request					\$2,500,000

Program Director/Principal Investigator (Last, First, Middle): Khan, Ikhlas, A.

BUDGET JUSTIFICATION

GRANT NUMBER
1U01FD004246

Provide a detailed budget justification for those line items and amounts that represent a significant change from that previously recommended. Use continuation pages if necessary.

A. PERSONNEL: \$1,241,419

Faculty and Professional Staff

PI, Dr. Ikhlas A. Khan, Assistant Director and Research Professor, National Center for Natural Products Research, Principal Investigator will devote 45% of his time to this program. Dr. Khan will be the contact point with NCNPR for interactions with FDA scientists. Dr. Khan has extensive research project administrative experience, has served as PI of several projects. Dr. Khan is currently working as a program director of FDA program at the Center. He works directly with Dr. Walker on a daily basis for scientific direction of major portions of NCNPR research efforts.

Co-Investigator, Dr. Larry A. Walker, Director, National Center for Natural Products Research, Co-Principal Investigator will provide the time & effort necessary for the overall administrative direction of the program. Dr. Walker will organize and coordinate the Steering Committee operation, and work closely with FDA officials and University administration to ensure administrative compliance and performance. No costs will be incurred to the grant for Dr. Walker's support.

CURRENT BUDGET PERIOD

FROM
09/01/13

THROUGH
08/31/14

Explain any estimated unobligated balance (including prior year carryover) that is greater than 25% of the current year's total budget.

N/A

BUDGET JUSTIFICATION CONTINUATION

Faculty and Professional Staff Continued

Principle Research Scientist (Dr. Shabana I. Khan) - 23% effort. Dr. Khan will be responsible for direction of the efforts at UM to evaluate toxicological parameters for the natural products and botanical extracts. She will commit to the project. Dr. Khan's training is in biochemistry, and she has worked for many years in the screening development with natural products. She will supervise the efforts of the toxicology research associates.

Sr. Research Scientist, Synthetic Chemist (Dr. Amar Chittiboyina) – 50% effort. Dr. Chittiboyina will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards. Dr. Chittiboyina will be responsible for all scientific aspects of data management for the project. Dr. Chittiboyina will coordinate particularly with the botanists, geneticist, analytical and isolation chemistry investigators, as well as with FDA scientists involved in the project, to develop and modify the data management workflow.

Sr. Research Scientist, Analytical Chemist. (Dr. Bharathi Avula) – 70% effort. Research Scientist, National Center for Natural Products Research will be responsible for analytical method development. Dr. Avula is a trained pharmacist and analytical chemist. She has developed expertise over the years in analysis of botanicals. She will also be supervising Dr.'s Yan Hong Wang, and Mei Wang in developing relevant analytical methods.

Senior Research Scientist, Analytical Chemist. (Dr. Yan Hong Wang) – 100% effort. Dr. Wang will be responsible for analytical method development. Dr. Wang is a natural products chemist with analytical background. He has developed an extensive expertise over the years in the analysis of botanicals.

Research Scientist, Plant Genetics (Dr. Natscha Tehen) – 100% effort. Dr. Tehen will be responsible for plant genetics approaches applied to the authentication of botanical dietary supplements. She is experienced in all the molecular techniques needed to accomplish this task.

Research Scientist, Isolation Chemist (Dr. Zulfiqar Ali) – 25% effort. Dr. Ali will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Research Scientist, Biologist (Dr. Gouyi Ma) – 100% effort. Dr. Ma will be responsible for the development of in-vitro assays to assess the toxicological profile of botanicals

Research Scientist, Chemist – (Ahmad Osman) 100% effort. This individual will be responsible for isolating and identifying compounds of interest from various botanical sources. Additionally, this person will be instrumental in compiling scientific information with regard to identification techniques (analytical, NMR, etc) for the authentication of botanical dietary supplements for the purpose of populating a dynamic web portal to assist FDA researchers and other stakeholders.

Associate Research Scientist, Isolation Chemist (Dr. Jiaping Zhao) – 53% effort. Dr. Zhao's responsibility will be to prepare extracts of selected botanicals, develop NMR identification/authentication techniques, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Postdoctoral Research Associate, Botanist (Dr. Vijayasankar Raman) – 100% effort. Dr. Raman will be working on macro and microscopy of botanicals, needed for species authentication. Microscopy provides an understanding of anatomical features of a plant, which is instrumental in differentiating the species and also in determining potential adulterants.

Program Director/Principal Investigator (Last, First, Middle): Khan, Ikhlas, A.

Post Doctoral Research Associate, Analytical Chemist (Dr. Mei Wang) – 70% effort. Dr. Wang will develop analytical methods for the purpose of identifying botanical dietary supplement samples. Dr. Wang has several years of experience in developing analytical GC techniques for the purpose of analysis and quantitation.

Post Doctoral Research Associate, Chemist (Dr. Cristina Avonto) – 100% effort. Dr. Avonto will be responsible for isolating marker compounds and bioactive constituents from botanicals. Additionally Dr. Avonto will perform analytical profiling of botanicals using various GC techniques.

Post Doctoral Research Associate, Biologist (Dr. Vamshikrishna Manda) – 100% effort. Dr. Manda will be responsible for the development of in-vitro assays to assess the safety of dietary supplement ingredients as well as ADMET evaluation of various constituents.

Post Doctoral Research Associate, Isolation Chemist (Zhihao Zhang) – 100% effort. Dr. Zhang will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Post Doctoral Research Associate, Isolation Chemist (Dr. Min Hye Yang) – 100% effort. Dr. Yang will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Assoc. R&D Biologist (Helaina Craig) – 100% effort. Ms. Craig will help the senior scientists on animal based in vivo work for behavioral and hepatotoxic studies on the botanical of interest.

Post Doctoral Research Associate, Isolation Chemist (Dr. Satyanarayanaraju Sagi) – 100% effort. Dr. Sagi will develop analytical methods for the purpose of identifying botanical dietary supplement samples. Dr. Sagi has several years of experience in developing analytical HPTLC/LC techniques for the purpose of analysis and quantitation.

Post Doctoral Research Associate, Isolation Chemist (Dr. Pradeep Lasonkar) – 100% effort. Dr. Lasonkar will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Post Doctoral Research Associate, Plant Genetics (Iffat Parveen) – 100% effort. Dr. Parveen will be responsible for plant genetics approaches applied to the authentication of botanical dietary supplements. She will assist and train under Dr. Techen for the molecular techniques needed to accomplish the proposed work.

Post Doctoral Research Associate, Analytical Chemist (Amira Wanas) – 50% effort. Dr. Wanas will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

R&D Botanist (Lal Jayaratna) – 100% effort. This individual is responsible for maintaining the living collection of the centers medicinal plant garden. Additionally this individual prepares voucher specimens, maintains the programs seed bank and provides assistance/tours for training sessions and workshops.

R&D Data Analyst – (Steven Hopper) 100% effort. This individual will be responsible for the development of a botanical dietary supplement information portal for the dissemination of relevant scientific data and FDA Dietary Supplement cGMP information. In addition to this he will be aiding in the data labeling, collection/reporting efforts for this project.

Project Coordinator (Gray Dale) - 25% effort. Mr. Dale is responsible to the PIs, to allow for adequate follow-up with reports, budgets and travel. Mr. Dale also provides vital logistical support for ICSB conference.

Technical and Administrative Staff

Program Coordinator (Jennifer Taylor) - 100% effort. Ms. Taylor is responsible to the PIs, to allow for adequate follow-up with reports, publications, file documentation, sample tracking, etc. Ms. Taylor also provides vital logistical support for workshops, training sessions and conferences.

NOTE: The position of Program Coordinator and Project Coordinator is normally not allowed as direct costs under OMB circular A-21. However, we are requesting these direct costs be allowed due to the large scope of the project and the number of personnel to be managed and supported. This position is easily allocable to the project, and are reasonable given the size and nature of the project.

Hourly Wages – Hourly wage support (\$17,000) will be utilized for temporary and student workers employed in support of the project. These may be utilized in support of administrative or research activities, ranging from filing and data entry to field/greenhouse or laboratory assistance.

Graduate Students (4) – The graduate students will receive training in natural products chemistry, analytical chemistry. These students will be enrolled in the Ph.D. program in the Department of Pharmacognosy, or one of the other academic Departments in the school of Pharmacy (\$40,000).

Fringe Benefits

Fringe benefits for faculty and staff are calculated at the University's standard rate of 32.75% of salary. Fringe benefits for students (graduate or undergraduate) are calculated at the University's standard rate of 8% of wages.

Increase for additional Years:

Inflationary increases of 3% per year have been included for year for personnel positions

B. CONSULTANT COSTS: None

C. EQUIPMENT: \$150,000

These funds are to support the capacity for analytical work that is required for the success of the program. These funds will be utilized to either purchase new complete systems or to upgrade other existing equipment such as HPLC, GC, NMR or MS.

D. SUPPLIES: \$ 165,181

Primary commodity expenditures for the project will be for:

HPLC columns \$22,947

NMR/MS supplies (tubes, gases, columns) \$13,700

Microscopic supplies (slides, stains, optics, mounting preparation) \$6,700

Chromatographic supplies (TLC plates, chromatographic media, solvents) \$50,834

Mol. Biology supplies \$30,000

Botanical collection/storage materials \$13,000

Garden/greenhouse tools/supplies \$12,000

Books, databases other reference materials \$12,000

Computer supplies \$4,000

Sub Total: \$165,181

E. TRAVEL: \$40,000

Included in this category are:

Travel for FDA Dietary Supplement GMP training sessions.

Establishing collection arrangements and collaborations (National and International)

Travel to/from Washington or other COE's for meetings with FDA officials

Travel costs related to hosting workshop/conference (National and International)

F. CONTRACTUAL SERVICES: \$140,000

Estimated expenditures in this category include contractual/professional services for:

Collection & documentation of plant material \$9,000

scale-up extraction/isolation \$ 9,000

taxonomic verifications \$6,000

maintenance contracts/repairs for analytical equipment \$39,500

software/upgrades for analytical equip. \$8,000

shipping, mailing costs \$4,000

Sub-Total: \$75,500

Estimated expenses for hosting conference:

Printing/PR \$3,500

Speaker reimbursements (28 @ 1,500) \$42,000

Dinners/breaks \$11,000

Staffing \$8,000

Sub Total: \$64,500

G. SUBCONTRACT: \$54,296

A subcontract with Missouri Botanical Garden will be in place in the amount of \$54,296. Missouri Botanical Garden will be collecting the specimens for this project and will be providing expertise on identification of the specimens. Dr. Rainer Bussmann, the director and curator of the William L. Brown Center for Plant Genetic Resource (WLBC), will be the Missouri Botanical Garden representative working on the project.

H. INDIRECT CHARGES: \$ 709,104

Indirect costs are calculated in accordance with The University of Mississippi's rate agreement with DHHS, dated September 12, 2011. Indirect costs for research are calculated at 44.0% of Total Direct Costs less equipment, tuition remission, and the portion of each sub-grant or subcontract in excess of \$25,000.

Program Director/Principal Investigator (Last, First, Middle): Khan, Ikhlas A

PROGRESS REPORT SUMMARY	GRANT NUMBER 1U01FD004246	
	PERIOD COVERED BY THIS REPORT	
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Khan, Ikhlas A	FROM 09/1/2013	THROUGH 08/31/2014
APPLICANT ORGANIZATION The University of Mississippi		
TITLE OF PROJECT (Repeat title shown in Item 1 on first page) Science Based Authentication of Dietary Supplements		

A. Human Subjects (Complete Item 6 on the Face Page)

Involvement of Human Subjects ☒ No Change Since Previous Submission ☐ Change

B. Vertebrate Animals (Complete Item 7 on the Face Page)

Use of Vertebrate Animals ☒ No Change Since Previous Submission ☐ Change

C. Select Agent Research ☒ No Change Since Previous Submission ☐ Change

D. Multiple PD/PI Leadership Plan ☒ No Change Since Previous Submission ☐ Change

E. Human Embryonic Stem Cell Line(s) Used ☒ No Change Since Previous Submission ☐ Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

None

Progress Report Summary

A. Specific Aims

Under the provisions of the 20 years of DSHEA, the FDA has primary responsibility for ensuring that appropriate regulatory actions are taken against marketed products that present significant health risks or bear false or misleading label claims. Foundational to the evaluation of such risks and claims is an adequate scientific base for decision-making. For botanical dietary supplements (BDS), development of such a science base is especially problematic because of several unique features, including the complexity of the constituents, variability of sourcing, lack of availability of reference materials, lack of good manufacturing controls and rapidly expanding use in the marketplace. In 2010 the FDA fully implemented the current good manufacturing practices (cGMP) for the botanical dietary supplement industry placing the onus of "botanical identity and authenticity" on the manufacturers of botanical dietary supplements. However, these cGMP's have in many ways increased the complexity as to what constitutes a "scientifically valid method" for the determination of the identity and authenticity of botanical materials. To address these complex issues surrounding botanical identity, authenticity, safety and toxicity and more importantly, in continuation of our cooperative agreement with the FDA, the NCNPR has established the following specific aims to facilitate the research needs of the FDA:

1. Assist in the identification and development of a list of BDS and botanical ingredients based on safety concerns, trends, and knowledge of botanicals being marketed in the U.S., to prioritize for further research.
2. Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to FDA, for the assessment of their adulteration, safety and toxicity.
3. Exchange technical and scientific information, analytical methods, and reference material with FDA scientists and other stakeholders.
4. Collaborate with FDA scientists in research areas of mutual interest.
5. Coordinate scientific workshops and conferences on BDS-related topics of public health relevance to address high priority science and awareness of emerging problems associated with botanicals to the public.

B. Studies and Results:

Aim 1: Assist in the identification and development of a list of BDS and botanical ingredients based on safety concerns, trends, and knowledge of botanicals being marketed in the U.S., to prioritize for further research.

Since the inception of the cooperative agreement between the NCNPR and the FDA in 2001, there have been numerous occasions for interactions between the two organizations. These have included (yearly) formal site visits by the FDA program officers, interactions during the annual International Conference on the Science of Botanicals (ICSB) and several conference calls and email exchanges. In addition to these interactions, over the past year the NCNPR has hosted five training sessions with the Office of Regulatory Affairs (ORA) to provide hands-on training to FDA inspectors for cGMP compliance issues associated with BDS's (FD340). These training sessions have provided an opportunity for the programs project officer (Dr. Daniel Fabricant) and his colleagues to visit the NCNPR and stay abreast of the Center's ongoing developments. As always, researchers at the NCNPR are receptive to areas of study that are deemed necessary by the FDA for this project.

Aim 2: Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to FDA, for the assessment of their adulteration, safety and toxicity.

The availability of authenticated reference materials for botanicals is an essential first step in the development of analytical methodologies for the identification and quantitative analysis of botanical product(s). The NCNPR has been very successful in placing formal agreements with several international academic and governmental

institutions including Pakistan, India (FRLHT), South Africa, Central/South America, China with the development of the Sino-US TCM Research Center and the established Center for Research in Indian Systems of Medicine (CRISM - www.CRISM.net) with the departments of AYUSH and CSIR in India. The NCNPR has also established a Memorandum of Understanding (MOU) with the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Brazil and the Natural Products Utilization Research Unit (NPURU), USDA located in Oxford Mississippi. Recently the NCNPR has cultivated a productive relationship with the Chinese Pharmacopeia and Chinese FDA in order to obtain relevant authenticated Traditional Chinese Medicines (TCM's) and pertinent purified constituents for authentication purposes. With this mechanism, NCNPR has acquired more than 150 new constituents over the past year and the collaboration would facilitate NCNPR to aid in populating a botanical information portal for CFSAN/FDA and expanding the in-house repository. In 2013, as a part of collaboration, more than 125 samples of authenticated tea tree oils were obtained from Southern Cross University, Australia to assess the safety, development of authentication techniques and to provide samples for possible allergen testing for CFSAN's cosmetic program. Lastly, NCNPR established an agreement with Tshwane University of Technology, Pretoria, South Africa to exchange the traditional practices based on botanicals endemic to South Africa such as *Sutherlandia frutescens*, *Hoodia gordonii* and other related plant materials of interest to the FDA.

From these and other collaborative relationships, the NCNPR has been able to acquire over 9500 plant samples and herbal extracts, representing approximately 5000 species over the duration of this project. There is a continuous effort in acquiring commercial samples as well for authentication purposes in addition to assuring that the developed identity testing techniques for this project are applicable for finished products in various formularies. Taking all these sample types into account, there are over 16,000 samples within the NCNPR-FDA repository. All the plants and samples collected for this program have been assigned an inventory number and are cataloged in the NCNPR repository and voucher specimens of authentic samples are also maintained at the Thomas M. Pullen Herbarium in the Department of Biology at the University of Mississippi.

In addition to the repository, the NCNPR has a newly renovated Medicinal Plant Garden that maintains more than 300 species for selected growing (field, greenhouse and shade houses). The new facilities consist of two main buildings (4,362 sf. and 4,290 sf.) and four additional support buildings and structures sitting on approximately 5.25 acres of land. The new facility was dedicated as the Maynard W. Quimby Medicinal Plant Garden on April the 15th 2013 as a part of the 12th ICSB. The Medicinal Plant Garden has developed significant collaborations with seed banks from 40 institutes around the world in order to exchange medicinally important germplasms. This seed bank effort has yielded a collection of over 1530 species to date. In addition, the garden personnel are preparing herbarium voucher specimens from the living collection and have started collecting samples for a DNA tissue bank. Over the course of this program, the garden provided 320 authentic reference samples from the living collection for the Center's research efforts. The Medicinal Plant Garden is also registered under the Botanic Gardens Conservation International and is a member of the American Association of Botanical Gardens and Arboreta and International Plant Exchange Network (IPEN). Overall, this new facility provides not only an invaluable resource for propagating and sourcing botanicals of interest but also provides a training facility for FDA/ORA courses on identification of botanical of interest.

Isolation of reference compounds: The isolation of standard compounds is required to develop analytical methods, quantify individual compounds in extracts and to establish analytical fingerprints in support of authenticity, quality, safety, and toxicity studies. Scientists at the NCNPR have isolated a number of compounds from species such as *Mitragyna speciosa*,¹ *Dioscorea villosa*,² *Dioscorea cayenensis*,³ *Dioscorea nipponica*,⁴ *Matricaria recutita* L., *Anthemis nobilis*, and *Lepidium meyerii*⁵ (Maca). It is through these continued efforts that the NCNPR scientist have isolated several novel compounds as well as known analogs for analytical finger printing development for authentication purposes as well as safety analysis.

Synthesis and procurement of compounds of interest: Under certain situations, synthesis of reference compounds is also undertaken at NCNPR wherein isolation of marker compounds was laborious and time consuming. Several sympathomimetic amines such as *N,N*-diethylphenethylamine, *N,N*-dimethylphenethylamine, phenethylamine, cocularine were synthesized from commercially available raw

materials on a bulk scale. In addition to large scale synthesis, several single enantiomers were synthesized for the development of analytical methods to understand the origin (synthetic/natural) of compounds of interest.

Analytical method development and metabolomic profiling: In the past year we have focused on developing several analytical methods for the determination of authenticity BDS's of interest. These studies include the development of UPLC (UV, ELS and MS), Supercritical Fluid Chromatography (SFC), standard HPLC/HPTLC analytical methods as well as using proton NMR for metabolomic profiling for common botanicals including *Matricaria recutita* L., (German chamomile) *Anthemis nobilis*, (Roman chamomile), *Chrysanthemum morifolium* (Chinese chamomile); determination of coumarin in Cinnamon species⁶ (*Cinnamomum verum*, *C. cassia*, *C. loureiroi*, and *C. burmannii*); *Terminalia* species⁷⁻⁹; *Dioscorea* species (*Dioscorea villosa* L., *D. cayennensis* Lam., *D. rotundata*, *D. opposita*, *D. caucasica*, *D. bulbifera*, *D. deltoidea*, *D. quaternata*); *Mitragyna speciosa*^{10, 11} Korth; *Dendrobium nobile*; pyrrolizidine alkaloids from *Asteraceae*, *Boraginaceae*, *Fabaceae*; multifarious skin whitening agents; estimation of glucose¹² and steviol glycosides; *Pelargonium graveolens*; *Serenoa repens*; and *Prunus africana*. Most importantly, the Center aided in developing an analytical approach establishing the absence of dimethylamylamine (DMAA) in authenticated *Pelargonium graveolens*. This newly developed method provided the FDA with the information required to challenge the marketing of DMAA products for lack of safety evidence on April 27, 2012. The results of this study were used to support FDA's position that DMMA found in dietary supplements sold in the U.S. (at >1 mg/g) must be synthetic. This finding led to the issuance by the agency of 10 warning letters to manufacturers and distributors of dietary supplements containing DMMA for which evidence of the safety of the product had not been submitted to FDA. The agency furthered warned the companies that synthetically-produced DMMA is not a "dietary ingredient" and, therefore, is not eligible to be used as an active ingredient in a dietary supplement.

Aim 3: Exchange technical and scientific information, analytical methods, and reference material with FDA scientists and other stakeholders.

Dr. Ikhlas Khan, Scientific Director for the project, has been in touch regularly with Dr. Daniel Fabricant (CFSAN, Director, Division of Dietary Supplement Programs), Dr. Elizabeth Calvey (CFSAN liaison), Dr. Diego Rua (CFSAN) and Dr. Robert L. Sprando, (CFSAN/OARSA, Director, Division of Toxicology) to exchange information on developed methods, reference materials availability, safety evaluations and project direction. These meetings and calls are designed to provide these individuals with an update of the NCNPR's overall program and to gain insight as to how this Center can diligently address the needs of the FDA.

Additionally, the NCNPR FDA-COE has also continued to assist the FDA's Office of Regulatory Affairs (ORA) in training inspectors for cGMP compliance for BDS's (FD340). Researchers at the NCNPR provided four one-day workshops on botanical dietary supplement authentication techniques to 135 trainees and FDA officials on May 20th, 2013, June, 19th, 2013, April 9th, 2014, May 7th, 2014 and scheduled two one-day workshops on, June 25th, 2014 and September 10th, 2014. The main training course is held in Memphis, Tennessee so that the trainees can attend a one-day excursion to the NCNPR for a combination of lectures and laboratory courses and training sessions to see what authentication techniques can be implemented for BDS's. The course covered current techniques utilized to identify botanical materials (Microscopy, Taxonomy, Macroscopy, TLC, HPLC, UPLC, GC, CE, etc.) and was presented by Dr. Khan and colleagues at the NCNPR and included two one and one half hour lab courses on Analytical Methods, Botanical Authentication, and Nomenclature. It is anticipated that this training program will continue for the foreseeable future and will provide the FDA cGMP botanical dietary supplement inspectors a valuable tool that will assist in their ability to carry out their duties.

Researchers at the NCNPR have also provided their expertise in other training offered by the FDA/ORA/DHRD. One such course was an advanced level course for analysts who are performing regulatory sample analysis using mass spectrometry techniques for identification and authentication (LB 403). Specifically, Dr. Yan-Hong Wang provided a lecture to several FDA trainees on August 28th– 29th 2013 covering the topic of how mass spectroscopy can be utilized for the authentication of botanicals. As an extension of this joint training with JIFSAN, the NCNPR sent Dr. Suman Chandra to provide four presentations at a GAP & GMP workshop aimed at supply chain management for spices and botanical ingredients for the Indian spice board (September 17th–21st, 2012, Kochi, India). Dr. Chandra covered topics such as harvesting

considerations, transportation/processing, cleaning and sanitation techniques. This workshop was then expanded into a multi-day multi-site visit for several members of the Indian spice board to provide these individuals with onsite training and lectures to provide advanced GAP and GMP techniques. This workshop, entitled "Food Safety and Supply Chain Management for Spices and Botanical Ingredients" was hosted by JIFSAN from March 25th - April 2nd then the NCNPR from April 3rd - 5th 2013. Dr. Ikhlas Khan attended the Spices Board India and All India Spice Exporters Forum, organized the World Spice Congress (WSC) in Cochin on February 16th-19th 2014. The highlight of the Congress was the Theme 'Sustainability and Food Safety' which is very relevant and crucial to the current scenario in the food sector. The business sessions planned by the Congress was led by globally renowned industry experts and addressed the topics on sustainable agriculture programs and practices based on real time experiences, infrastructural development, harmonization and simplification of standards etc. He also used this time to discuss the current collaboration between JIFSAN and the Indian Spice Board. In addition to above scientific activity, Dr. Khan and Dr. Troy Smillie participated in the 3rd Annual FDA Foods and Veterinary Medicine Science and Research Conference held in College Park, MD on August 27th -28th, 2013.

Lastly, the research effort initiated by the establishment of this Center has provided several opportunities to leverage the existing cooperative agreement so as to expand the impact and overall benefit of the existing program. This includes a recently funded NCCAM/ODS Botanical Research Center grant to explore Botanical Estrogens: Mechanisms, Dose and Target Tissues (PD: Helferich, William G., University of Illinois/PL: Khan, Ikhlas, A., Award number 5 P50 AT006268-02). Under this grant the NCNPR is providing significant quantities and populations of authenticated samples of Licorice - *Glycyrrhiza glabra* Linné var *glabra*, and Wild Yam - *Dioscorea villosa* L., for the established BRC.¹³ In addition to obtaining the outlined authenticated species for this program, we have established a substantial growing and collection program for closely related species for authentication and comparative purposes. Additionally, the NCNPR has obtained funding from the USDA/ARS for a grant entitled Discovery and Development of Natural Product-Based Insect Management Compounds for Medicinal, Veterinary and Urban Concern, award number 58-6402-1-612. The purpose of this grant is to discover and develop new, safer and more effective insect management products derived from natural phytochemical sources.

Aim 4: Collaborate with FDA scientists in research areas of mutual interest.

Over the past year the NCNPR has provided the FDA with scientific information for botanicals of concern as well as investigated several plants that have purported adverse events. Most notably we have undertaken an investigation of "chamomile" which has had reports of contact dermatitis and potential severe cases of allergic reactions in cosmetic formulations. There are two main species of "chamomile" utilized for commerce within the United States German chamomile, *Matricaria recutita* L.¹⁴ and Roman chamomile, *Anthemis nobilis*. Typically the flowering tops are used for most cosmetic formulations and these are either added as powdered material or an extract (ethanolic, supercritical or steam distilled). Working closely with scientists in the FDA/CFSAN office of cosmetics and colors, we initiated an extraction and bioassay guided fractionation of both German and Roman chamomile utilizing an LLNA screening assay for lead identification. Initial results are indicated that there is a potential sensitizer(s) within *Matricaria recutita* L. that could be causing the purported adverse events. Simultaneously, scientists at NCNPR developed two complementary *in chemico* (non-biological, non-animal) methods for identification and classification of chemical compounds as potential skin sensitizers, using either Nuclear Magnetic Resonance (NMR) spectroscopy or High Throughput Spectrophotometric methods. Further investigation including isolation, purification and *in chemico* evaluations indicated that the photo-oxidative metabolite of tonghaosu as a potential sensitizer in *Matricaria recutita* L. Scale-up, *in vivo* confirmation with LLNA screening assay have been undertaken and are still in progress.

A second project undertaken for CFSAN's office of cosmetics and colors looked at products that include an essential oil known as "Tea tree oil", which is obtained from several species of *Melaleuca* plants. One general research objective for this project is to seek further knowledge on compositional differences between commonly used species of *Melaleuca* plants (*M. alternifolia*, *M. linariifolia*, *M. viridiflora*, *M. leucadendron*, *M. dissitiflora*, etc.) in order to explore ways to address safety concerns for these species. The main safety concern about essential oils from these plant species is the potential for adverse effects on the skin, in

particular sensitization or allergic contact dermatitis (ACD). It is not fully understood which of the tea tree oil constituents is responsible for ACD. Therefore, the potential ACD active(s) in tea tree oil are currently being identified utilizing recently developed in-house NMR and HTS screening methods. Concurrently, we have developed an analytical GC/MS method to differentiate between the various species of tea tree oil that also identifies the major constituents. This newly developed method can be used to help identify potential ACD's within products.

NCNPR established two in-house preliminary *in-vivo* safety evaluations for botanicals of interest. Priority evaluation was given to botanicals that have displayed an inherent risk based on literature information or reported adverse events. Initial screens focused on two areas of concern. The first mouse model measured botanicals for their potential to induce positive reinforcement or cause aversive properties using a conditioned place preference (CPP) paradigm procedure that is commonly used to evaluate drugs for their "addictive" behavior. Over the past year the Center used this model to evaluate *Salvia divinorum*, *Mitragyna speciosa*, *Sceletium tortuosum* and fractions and pure compounds isolated from these species. Scientists at NCNPR employed the place preference/aversion paradigm to characterize the psychoactive properties of *Salvia divinorum* ext. (10, 30, 100 mg/kg), salvinorin A (0.1, 0.3, 1.0 mg/kg), *Mitragyna speciosa* MeOH ext. (50, 100, 300 mg/kg), *Mitragyna speciosa* alkaloid-enriched fraction (12.5, 25, 75 mg/kg) and mitragynine (5, 10, 30 mg/kg) in rats. For *S. divinorum* the preliminary results indicated that this particular botanical did not induce abusive potential. For *M. speciosa* the results indicated that the major pharmacologically active constituent, mitragynine, has an abuse potential.¹⁵ Moreover, we have undertaken to study ADME properties of these compounds and their effect on the major efflux transporter P-glycoprotein, using in vitro methods. The stability of major alkaloids, mitragynine, 7-hydroxymitragynine and mitraphylline were subjected to Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF).¹⁶ All three compounds exhibited high plasma protein binding (> 90%) determined by equilibrium dialysis. Mitragynine and 7-hydroxymitragynine inhibited P-glycoprotein with EC₅₀ values of $18.2 \pm 3.6 \mu\text{M}$ and $32.4 \pm 1.9 \mu\text{M}$, respectively, determined by the calcein-AM fluorescent assay, while no inhibition was seen with mitraphylline. These data suggest the possibility of a drug interaction if mitragynine and 7-hydroxymitragynine are co-administered with drugs that are P-glycoprotein substrates.

As a part of phytochemical investigation, we have isolated several known and unknown alkaloids from *S. tortuosum*. Noticeably, these marker components will assist us in authentication, identification and development of analytical methods for *S. tortuosum* and its principle alkaloid components. The majority of these findings have not yet been published; however, they will be reported shortly. Along with ADME properties and intravenous plasma pharmacokinetics, the behavioral studies associated with effects of *Sceletium tortuosum* in rats are still in progress and the results will be reported in due course.

The second *in-vivo* assay was developed to evaluate the potential hepatotoxicity's associated for botanical as measured in a mouse model where the hepatotoxicity is manifested by elevation of aminotransferases and histopathological features of acute hepatocellular necrosis and/or biliary injury. Initial *in-vivo* hepatotoxicity evaluations of EGCG,¹⁷⁻²⁰ a major component in green tea products, at high doses can lead to mild liver injury and under febrile conditions can cause severe liver injury. Currently, we are testing hepatotoxicity potential of OxyElite Pro and Black Cohosh in mice and results will be reported in due course. Both *in-vivo* models will continue to provide significant insight into the safety profile for botanicals that are of concern to public health.

Lastly, NCNPR has provided the FDA (CFSAN/OARSA) with scientific information for botanicals of concern as well as investigated several plants that are purported to have hepatotoxicity. Working closely with Dr. Sprando and his colleagues' at OARSA, we identified several sympathomimetic compounds and extracts reported to have hepatotoxic potential. Based on their usage in BDS, five whole methanolic extracts of *Astragalus membranaceus*, *Rauvolfia serpentina*, *Calea zacatechichi*, *Psoralea corylifolia*, *Adhatoda zeylanica*, *Kigelia Africana* were provided to OARSA to estimate the potential toxicity. In addition to these extracts, nine pure compounds were also provided to OARSA. Of nine pure compounds, based on practicality and other factors, four compounds, *N,N*-diethylphenethylamine, *N,N*-dimethylphenethylamine, phenethylamine, cocularine were further selected for animal studies. At this point, 5.0 Kg of cocularine was provided to OARSA and the findings will be reported in due course.

Aim 5: Coordinate scientific workshops and conferences on BDS topics of public health relevance to address high priority science and research needs.

The Center hosted the 13th Oxford International Conference on the Science of Botanicals (ICSB) that was held on April 15th – 17th, 2014, at The University of Mississippi to commemorate the 20 years of DSHEA. In addition to regulatory aspects with perspectives from government, manufacturers and trade associations; post market surveillance, risk and safety assessment, quality control and adverse event reporting (AER) for botanical dietary supplements (BDS) and natural products were discussed at 13th ICSB. This conference was co-sponsored by the Shanghai Institute of Materia Medica/CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant and Natural Products Research (GA); the Korean Society of Pharmacognosy and the Vietnam Academy of Science and Technology (VAST) the St Petersburg Institute of Pharmacy; the Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD) and the International Council on Medicinal and Aromatic Plants for Human Welfare (ICMAP) as such included representative delegations of scientists from various organizations in China, India and Europe. Due to the focused scheduling of the 13th ICSB, contributed presentations or poster submissions were not accepted. These types of presentations are reserved for the upcoming 2014 ASP/14th ICSB (<http://asp2014.org/>) which will be hosted in August 2nd - 6th 2014 Oxford, MS. This conference will cover the topics of TCM, Ayurveda, cultivation, collection, post-harvest, processing practices, identification, authentication and quality/safety assessment of botanicals including current clinical evaluations, regulatory aspects and the impact on the global dietary supplement industry. Again the proceedings from these conferences are expected to be published in *Planta Medica*.

C. Significance:

Plant collection, authentication, voucher specimens, isolation, synthesis of reference compounds and method development provide the basic tools for assessing the safety and quality of botanical products. Significant progress has been made by the NCNPR in each of these areas for several botanicals of interest over the past year. The acquired information, physical samples (plants, extracts, etc.) and phytochemical standards are freely available to researchers at the FDA for the evaluation purposes. Publications from this project have significantly contributed to the available methodological literature for the FDA and NCNPR (see references).

D. Plans:

To address the needs of the FDA on safety of BDS, Dr. Ikhlas Khan, Scientific Director for the project, would be in touch with Director, Division of Dietary Supplement Programs and liaison at CFSAN. The center will continue to exchange information on developed methods, reference materials availability, safety evaluations and project direction with CFSAN. Similar to DMAA, continual research effort will also focus on presence/absence of several alkaloids of concern for their potential safety concern due to their abuse potential, undesired adrenergic, dopaminergic receptor activities. For example, hydrastine, berberine, berberastine, hydrastinine, tetrahydroberberastine, canadine, and canalidine from *Hydrastis Canadensis*;²¹ yohimbine²² and related alkaloids from *Pausinystalia yohimbe* which is widely used as a supplement for bodybuilding and to enhance male sexual performance; Aegeline, several tetrahydroisoquinoline compounds such as boldine, reticuline and related compounds from *Aegle marmelos*.

Furthering the collaborative research effort we have established with CFSAN's office of cosmetics and colors, additional effort will continue to look at several potential areas of concern. The first being the continued exploration of products that contain "tea tree" essential oil(s) which can be derived from several species of *Melaleuca* plants. In addition to compositional differences between commonly used species of *Melaleuca* plants (*M. alternifolia*, *M. linariifolia*, *M. viridiflora*, *M. leucadendron*, etc.); the main research objective for this project will be to seek further knowledge on the sensitization potential of these essential oils using recently developed in-house in chemico methods and compare, validate the resulting data with KeratinoSens, direct peptide reactivity assay (DPR). At the same time, we will also explore the stability, aging, isolation and identification of possible reactive intermediate(s) in these oils using the recently developed GC-MS analytical method.

Continual research effort will also focus on the two recently developed in-house *in-vivo* screen evaluating botanicals for their potential to induce positive reinforcement or cause aversive properties using the developed CPP paradigm procedure that is commonly used to evaluate drugs for "addictive" behavior and the second assay which evaluates potential hepatotoxicity associated for certain botanicals. Priority evaluation will be given to botanicals that have displayed an inherent risk based on literature information or reported adverse events. Both models will provide significant insight as to the safety profile for botanicals that are of concern to the public health.

The PI and Scientists at NCNPR will continue to work with OARSA by exchanging the scientific information on botanicals of concern with hepatotoxicity potential. For animal studies purpose, on bulk scale, three other compounds, *N,N*-diethylphenethylamine, *N,N*-dimethylphenethylamine, phenethylamines would be provided to OARSA. By working closely with OARSA, the Center will continue to provide significant insight into the authentication, validation, analytical and safety profile for BDS that are of hepatotoxic concern to public health.

To collect the information from labels which can help in determining the quantity of any given dietary ingredient, a label database will be developed. This database will assist in exposure calculations and report generation for general ingredient risk assessment. This information will be used internally as well as for public good.

Lastly, expansion of the existing repository of reference plant samples for the genera noted throughout this report will be a continuing priority for this project. New botanicals will be selected based on input received from the FDA program officer, collaborators and liaison for further studies and to evaluate their safety and quality. A fifteenth Oxford International Conference on the Science of Botanicals (ICSB) is proposed to be held on April 13th – 16th, 2015, Oxford, MS. The conference will be co-sponsored by the Shanghai Institute of Materia Medica/CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the Ministry of Indigenous Medicine, Sri Lanka; the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant and Natural Products Research (GA); the Korean Society of Pharmacognosy and the Vietnam Academy of Science and Technology (VAST) the St Petersburg Institute of Pharmacy; the Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD) and the International Council on Medicinal and Aromatic Plants for Human Welfare (ICMAP). This conference will cover the topics of TCM, Ayurveda, cultivation, collection, post-harvest, processing practices, identification, authentication and quality/safety assessment of botanicals including current clinical evaluations, regulatory aspects and the impact on the global dietary supplement industry. Again the proceedings from this conference are expected to be published in *Planta Medica*.

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ALL PERSONNEL REPORT

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Always list the PD/PI(s). In addition, list all other personnel who participated in the project during the current budget period for at least one person month or more, regardless of the source of compensation (a person month equals approximately 160 hours or 8.3% of annualized effort). Use the following abbreviated categories for describing Role on Project:

- PD/PI*
- Co-Investigator
- Faculty
- Postdoctoral (scholar, fellow, or other postdoctoral position)*
- Technician
- Staff Scientist (doctoral level)
- Statistician
- Graduate Student (research assistant)
- Non-student Research Assistant
- Undergraduate Student
- High School Student
- Consultant
- Other (please specify)

If personnel are supported by a Reentry or Diversity Supplement please indicate such after the Role on Project, using the following abbreviations: RS - Reentry Supplement; DS - Diversity Supplement.

*Commons ID required for any personnel holding this Role on Project and for all individuals supported by a Reentry or Diversity Supplement. The Commons ID will be required in the future for all individuals with a graduate student, or undergraduate role. The Commons ID is strongly encouraged, but not required, for all other Project Personnel.

Use Cal (calendar), Acad, or Summer to enter months devoted to project.

Commons ID*	Name	Degree(s)	SSN (last 4 digits)	Role on Project	DoB (MM /YY)	Cal	Acad	Summer
IKHLAS	Ikhlas Khan	Ph.D.	(b) (6)	PI	(b) (6)	5.4		
LARRYWA LKER2004	Larry Walker	Ph.D.		Co-PI		1.2		
SKHAN	Shabana Khan	Ph.D.		Staff Scientist		2.76		
BAVULA	Bharathi Avula	Ph.D.		Staff Scientist		8.4		
YAN HONG	Yan Hong Wang	Ph.D.		Staff Scientist		12		
	Natasha Techen	Ph.D.		Staff Scientist		12		
ALI	Zulfiqar Ali	Ph.D.		Staff Scientist		3		
CHITTIBOY INA	Amar Chittiboyina	Ph.D.		Staff Scientist		6		
	Gouyi Ma	Ph.D.		Staff Scientist		12		
	Ahmad Osman	Ph.D.		Staff Scientist		12		
JPZHAO	Jianping Zhao	Ph.D.		Staff Scientist		6.36		
VRAMAN	Vijayasankar Raman	Ph.D.		Postdoc		12		
	Mei Wang	Ph.D.		Postdoc		8.4		

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- Postdoctoral (scholar, fellow, or other postdoctoral position)*
- Technician
- Staff Scientist (doctoral level)
- Statistician
- Graduate Student (research assistant)
- Non-student Research Assistant
- Undergraduate Student
- High School Student
- Consultant
- Other (please specify)

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Use Cal (calendar), Acad, or Summer to enter months devoted to project.

Commons ID*	Name	Degree(s)	SSN (last 4 digits)	Role on Project	DoB (MM /YY)	Cal	Acad	Summer
	Christina Avonto	Ph.D.	(b) (6)	Postdoc	(b) (6)	12		
	Amira Wanas	Ph.D.		Postdoc		6		
	Gray Dale	MA		RsCh Asst		3		
	Zhihao Zhang	Ph.D.		Postdoc		12		
	Pradip Lasonkar	Ph.D.		Postdoc		12		
	Iffat Parveen	Ph.D.		Postdoc		12		
	Vamshikrishna Manda	Ph.D.		Postdoc		12		
	Min Hye Yang	Ph.D.		Postdoc		12		
	Satyanarayanaraju Sagi	Ph.D.		Postdoc		12		
	Helaina Craig	BA		RsCh Asst		12		
	Lal Jayaratna	MSc		RsCh Asst		12		
	Steven Hopper	BFA		Technician		12		
	Jennifer Taylor			RsCh Asst		12		

From: Jones, Bryce
Sent: Tuesday, September 06, 2016 1:58 PM
To: Ikhlas Khan
Cc: research@olemiss.edu; Welch, Cara; Geathers, LaQuia; Brown, Tashea D
Subject: NEW, Notice of Grant Award (University of Mississippi) 2U01FD004246-06
Attachments: SKM_C654e16090613540.pdf

Importance: High

Good Afternoon,

Please find attached, the Notice of Grant Award for 2U01FD004246-06 for the University of Mississippi. If you should have any questions, please feel free to contact me.

Kind Regards,
Bryce Jones

Bryce Jones | Grants Management Specialist
U.S. Food & Drug Administration | Office of Acquisitions & Grants Services | Division of State Acquisitions, Agreements and Grants
5630 Fishers Lane, Room 2026, Rockville, MD 20857 | (240) 402-2111 | Bryce.Jones@fda.hhs.gov



FOOD AND DRUG ADMINISTRATION

Grant Number: 2U01FD004246-06
FAIN: U01FD004246

Principal Investigator:
Ikhlas Ahmad Khan, PHD

Project Title: Science based authentication of botanical ingredients

Dr. Buchannon, Robin C., Ph.D
Asst Vice Chancellor-Research&Sponsored Pgms
100 Barr Hall
P.O. Box 1848
University, MS 386771848

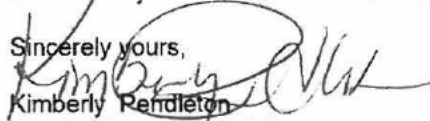
Budget Period: 09/05/2016 – 08/31/2017
Project Period: 09/15/2011 – 08/31/2021

Dear Business Official:

The Food and Drug Administration hereby awards a grant in the amount of \$2,158,000 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MISSISSIPPI in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 31 USC 6305 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

If you have any questions about this award, please contact the Grants Management Specialist and the Project Officer listed in the terms and conditions.

Sincerely yours,

Kimberly Rendleton
Grants Management Officer
Office of Acquisitions & Grants Services
Division of Acquisition Support and Grants
Grants & Assistance Team
FOOD AND DRUG ADMINISTRATION

See additional information below

SECTION I – AWARD DATA – 2U01FD004246-06**Award Calculation (U.S. Dollars)**

Salaries and Wages	\$692,371
Fringe Benefits	\$224,473
Personnel Costs (Subtotal)	\$916,844
Consultant Services	\$90,000
Equipment	\$80,000
Supplies	\$174,500
Travel Costs	\$48,000
Other Costs	\$114,500
Consortium/Contractual Cost	\$75,000

Federal Direct Costs	\$1,498,844
Federal F&A Costs	\$659,156
Approved Budget	\$2,158,000
Federal Share	\$2,158,000
TOTAL FEDERAL AWARD AMOUNT	\$2,158,000

AMOUNT OF THIS ACTION (FEDERAL SHARE)	\$2,158,000
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SUMMARY TOTALS FOR ALL YEARS			
YR	THIS AWARD		CUMULATIVE TOTALS
6		\$2,158,000	\$2,158,000
7		\$2,158,000	\$2,158,000
8		\$2,158,000	\$2,158,000
9		\$2,158,000	\$2,158,000
10		\$2,158,000	\$2,158,000

* Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project.

Fiscal Information:

CFDA Number:	93.103
EIN:	1646001159A1
Document Number:	UFD004246C
PMS AccountType	P(Subaccount)
Fiscal Year:	2016

IC	CAN	2016	2017	2018	2019	2020
FD	6991712	\$350,000	\$2,158,000	\$2,158,000	\$2,158,000	\$2,158,000
FD	6999BTC	\$1,808,000				

* Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project.

FDA Administrative Data:

PCC: CFS02 / OC: 4141 / Processed: ERAAPPS 09/01/2016

SECTION II – PAYMENT/HOTLINE INFORMATION – 2U01FD004246-06

Grant payments will be made available through the DHHS Payment Management System (PMS). PMS is administered by the Division of Payment Management, Program Support Center (PSC),

DHHS, Office of the Deputy Assistant Secretary, Finance. Requests for downloadable forms and inquiries regarding payment should be directed to:

Regular Mailing Address:
Division of Payment Management
P.O. Box 6021
Rockville, MD 20852
Telephone: (301) 443-1660

Included are the following Links & Instructions for drawing down funds, reporting expenditures, required forms, and the help desk info:

Homepage: <http://www.dpm.psc.gov/Default.aspx>

Grant Recipient Information:
http://www.dpm.psc.gov/grant_recipient/grant_recipient.aspx?explorer.event=true

Grant Recipient Forms:
http://www.dpm.psc.gov/grant_recipient/grantee_forms.aspx?explorer.event=true

PMS Help Desk: <http://www.dpm.psc.gov/help/help.aspx?explorer.event=true>

The ONE-DHHS Help Desk for PMS Support is now available Monday – Friday from 7 a.m. to 9 p.m. EST (except Federal Holidays). Phone (877) 614-5533; Email PMSSupport@psc.gov

SECTION III – TERMS AND CONDITIONS – 2U01FD004246-06

This award is based on the application submitted to, and as approved by, FDA on the above-title project and is subject to the terms and conditions incorporated either directly or by reference in the following:

- a. The grant program legislation and program regulation cited in this Notice of Grant Award.
- b. The restrictions on the expenditure of federal funds in appropriations acts to the extent those restrictions are pertinent to the award.
- c. 45 CFR Part 75.
- d. The HHS Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
- e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
- f. A required Federal Financial Report (FFR) SF-425 must be submitted annually. FDA now requires all annual financial expenditure reports to be submitted electronically using the Federal Financial Report (FFR) system located in the eRA Commons. Annual FFRs must be submitted for each budget period no later than 90 days after the end of the calendar quarter in which the budget period ended. The reporting period for an annual FFR will be that of the budget period for the particular grant; however, the actual submission date is based on the calendar quarter. Failure to submit timely reports may affect future funding.
- g. Closeout Requirements (when applicable): A Final Program Progress Activity Report, Final Federal Financial Report SF-425, Final Invention Statement HHS-568 (if applicable), Tangible Personal Property Report SF-428, and Statement of Disposition of Equipment (if applicable) must be submitted within 90 days after the expiration date of the project period.
- h. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

This award has been assigned the Federal Award Identification Number (FAIN) U01FD004246. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

**Treatment of Program Income:
Additional Costs**

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than \$10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75.

SECTION IV – FD Special Terms and Condition – 2U01FD004246-06

TERMS AND CONDITIONS

This award is based on the application submitted to, and as approved by, FDA on the above-title project and is subject to the terms and conditions incorporated either directly or by reference in the following:

- a. The grant program legislation and program regulation cited in this Notice of Grant Award.
- b. The restriction on the expenditure of federal funds in appropriations acts to the extent those restrictions are pertinent to the award.
- c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
- d. The PHS Grants Policy Statement including addenda in effect as of the beginning date of the budget period.
- e. This award notice, including the term and conditions cited below.

SPECIAL TERMS AND CONDITIONS

The following special terms of award are in addition to, and not in lieu of, otherwise applicable U.S. Office of Management and Budget (OMB) administrative guidelines, U.S. Department of Health and Human Services (DHHS) grant administration regulations at 45 CFR Part 75, and other HHS, PHS, and FDA grant administration policies.

The administrative and funding instrument used for this program will be the cooperative agreement, an "assistance" mechanism (rather than an "acquisition" mechanism), in which substantial FDA programmatic involvement with the awardees is anticipated during the performance of the activities. Under the cooperative agreement, the FDA purpose is to support and stimulate the recipients' activities by involvement in and otherwise working jointly with the award recipients in a partnership role; it is not to assume direction, prime responsibility, or a dominant role of activities. Consistent with this concept, the dominant role and prime responsibility resides with the awardees for the project as a whole, although specific tasks and activities may be shared among the awardees and the FDA as defined below.

A.1. Principal Investigator Rights and Responsibilities

The Principal Investigator will have the primary responsibility for and dominant role in planning, directing, and executing the proposed project, with the FDA staff being substantially involved as a partner with the PI.

For direct inquiries regarding scientific/programmatic issues or fiscal and/or administrative matters please refer to STAFF CONTACT below:

Grants Management Contact (financial and/or administrative concerns):

Bryce Jones
Grants Management Specialist
Food and Drug Administration, MSC HFA-500
5630 Fishers Lane, Rm 2026
Rockville, MD 20857
Phone: 240-402-2111
Email: Bryce.Jones@fda.hhs.gov

Programmatic Contact (technical and/or scientific concerns):

Cara Welch
Senior Advisor
CPK1 RM4D034 HFS-810
FDA College Park Campus - 5001 Campus Drive
College Park, MD 20740
Telephone: 240-402-2333
E-mail: Cara.Welch@fda.hhs.gov

Failure to comply with the above stated Program Terms and Conditions could result in the suspension or termination of this grant project.

THE EXPANDED AUTHORITIES DO NOT APPLY TO THIS GRANT

Direct inquiries regarding scientific programmatic issues to the official listed below.

Direct inquiries regarding fiscal and/or administrative matters to the grants management specialist listed below.

All formal correspondence/reports regarding the grant should be signed by an authorized institutional official and the Principal Investigator and should be sent to the attention of the grants management specialist, unless otherwise explicitly directed.

STAFF CONTACTS

Grants Management Specialist: Bryce Jones
Email: bryce.jones@fda.hhs.gov **Phone:** 240-402-2111

SPREADSHEET SUMMARY

GRANT NUMBER: 2U01FD004246-06

INSTITUTION: UNIVERSITY OF MISSISSIPPI

Budget	Year 6	Year 7	Year 8	Year 9	Year 10
Salaries and Wages	\$692,371	\$692,371	\$692,371	\$692,371	\$692,371
Fringe Benefits	\$224,473	\$224,473	\$224,473	\$224,473	\$224,473
Personnel Costs (Subtotal)	\$916,844	\$916,844	\$916,844	\$916,844	\$916,844
Consultant Services	\$90,000	\$90,000	\$90,000	\$90,000	\$90,000
Equipment	\$80,000	\$80,000	\$80,000	\$80,000	\$80,000

From: Pendleton, Kimberly
Sent: Wednesday, September 02, 2015 7:31 AM
To: Ikhlas Khan
Cc: research@olemiss.edu; Welch, Cara; CFSAN Awards Mailbox; Geathers, LaQuia
Subject: Notice of Grant Award (U01FD004246-05) Ikhlas A. Khan, PhD
Attachments: 2689_001.pdf

Good Morning Dr. Khan,

Please find enclosed your FY2015 Notice of Grant Award. If you should have any questions, please don't hesitate to contact me.

Best,
Kim
Kimberly Pendleton Chew, FDA
CGMO/Branch Chief



Notice of Grant Award

RESEARCH PROJECT COOPERATIVE AGREEMENT **Issue Date:** 09/01/2015
Department of Health and Human Services
FOOD AND DRUG ADMINISTRATION



Grant Number: 4U01FD004246-05
FAIN: U01FD004246

Principal Investigator:
Ikhlas Ahmad Khan, PHD

Project Title: Science Based Authentication of Dietary Supplements

Dr. Buchannon, Robin C.
Assistant Vice Chancellor for Research and Sp
100 Barr Hall
University, MS 386771848

Budget Period: 09/01/2015 – 08/31/2016
Project Period: 09/15/2011 – 08/31/2016

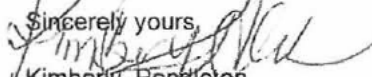
Dear Business Official:

The Food and Drug Administration hereby awards a grant in the amount of \$1,907,999 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF MISSISSIPPI in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 31 USC 6305 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

If you have any questions about this award, please contact the Grants Management Specialist and the Project Officer listed in the terms and conditions.

Sincerely yours,


Kimberly Pendleton
Grants Management Officer
Office of Acquisitions & Grants Services
Division of Acquisition Support and Grants
Grants & Assistance Team
FOOD AND DRUG ADMINISTRATION

See additional information below

SECTION I – AWARD DATA – 4U01FD004246-05**Award Calculation (U.S. Dollars)**

Other Costs	\$1,341,590
Federal Direct Costs	\$1,341,590
Federal F&A Costs	\$566,409
Approved Budget	\$1,907,999
Federal Share	\$1,907,999
TOTAL FEDERAL AWARD AMOUNT	\$1,907,999
AMOUNT OF THIS ACTION (FEDERAL SHARE)	\$1,907,999

SUMMARY TOTALS FOR ALL YEARS		
YR	THIS AWARD	CUMULATIVE TOTALS
5	\$1,907,999	\$1,907,999

* Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project.

Fiscal Information:

CFDA Number:	93.103
EIN:	1646001159A1
Document Number:	UFD004246B
PMS AccountType	P(Subaccount)
Fiscal Year:	2015

IC	CAN	2015
FD	6992030	\$1,907,999

* Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project.

FDA Administrative Data:

PCC: CFS02 / OC: 4141 / Processed: ERAAPPS 09/01/2015

SECTION II – PAYMENT/HOTLINE INFORMATION – 4U01FD004246-05

PHS policy requires that you be informed that the DHHS Inspector General maintains a toll free telephone number (800-368-5779) for receiving information concerning fraud, waste and abuse under the grants and cooperative agreements. Such reports will be kept confidential and callers may decline to give their names if they choose to remain anonymous.

Payments under this award will be made available through the DHHS Payment Management System (PMS). PMS is administered by the Division of Federal Assistance Financing (DFAF), Office of the Deputy Assistant Secretary, Finance, which will forward instructions for obtaining payments. Inquiries regarding the payment should be directed to:

Division of Federal Assistance Financing
DASP/DASF/OS/DHHS
P.O. Box 6021
Rockville, MD 20852
Telephone Number: 877-614-5533

Grantees are asked to register in the Central Contractor Registration (CCR) database. Information about CCR is available at http://www.grants.gov/applicants/register_ccr.jsp. This registration will be required as electronic grant processing is implemented.

SECTION III – TERMS AND CONDITIONS – 4U01FD004246-05

This award is based on the application submitted to, and as approved by, FDA on the above-title project and is subject to the terms and conditions incorporated either directly or by reference in the following:

- a. The grant program legislation and program regulation cited in this Notice of Grant Award.
- b. The restrictions on the expenditure of federal funds in appropriations acts to the extent those restrictions are pertinent to the award.
- c. 45 CFR Part 74 or 45 CFR Part 92 as applicable.
- d. The PHS Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
- e. An annual Financial Status Report (SF-269) is required. An original and two copies of this report must be submitted to the FDA Grants Management Officer within 90 days after the expiration date of the budget period.
- f. A Final Program Report, Financial Status Report and Invention Statement must be submitted within 90 days after the expiration date of the project period.
- g. This award notice, including the terms and conditions cited below.

This award has been assigned the Federal Award Identification Number (FAIN) U01FD004246. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

This award was issued as a non-competing continuation with a change in document number. This change was made solely to accommodate the HHS mandate to transition award payments to Payment Management System (PMS) subaccounts. Expenses for the project period should be treated as if this were a non-competing continuation award (e.g. Type 5).

**Treatment of Program Income:
Additional Costs**

SECTION IV – FD Special Terms and Condition – 4U01FD004246-05

THIS AWARD IS UNDER EXPANDED AUTHORITIES

TERMS AND CONDITIONS:

PLEASE NOTE: Grantee can rebudget funds to align with FY2015 funding.

This award is subject to the Special Requirements of the RFA Number FD11-004, entitled, "University of Mississippi's National Center for Natural Products Research (U01)" is hereby incorporated by reference as special terms and conditions of this award. Copies of this announcement may be obtained from the Grants Management Contact referenced in the award.

Please note as of October 1, 2006, the HHS Grants Policy Statement (GPS) supersedes in its entirety the above cited PHS GPS, dated April 1, 1994, and addendum dated January 24, 1995.

This award is subject to the requirements of the HHS Grants Policy Statement (HHS GPS) that are applicable to you based on your recipient type and the purpose of this award. This includes any requirements in Parts I and II (available at <http://www.hhs.gov/asfr/ogapa/aboutog/hhsgps107.pdf>) of the HHS GPS that apply to an award.

Although consistent with the HHS GPS, any applicable statutory or regulatory requirements, including 45 CFR part 74 or 92, directly apply to this award apart from any coverage in the HHS GPS that apply to an award.

PLEASE NOTE: Salary Cap: As of January 12, 2014, Salary Cap has changed to \$181,500. For FY2014 and beyond, grantees will need to rebudget salaries of an individual at a rate in excess of the current salary cap. Current salary cap level is \$181,500.

NOTICE: Human Subjects: Under governing regulations, no Federal funds administered by the Department of Health and Human Services may be expended for research involving human subjects or identifiable data, as defined by the Office for Human Research Protections (OHRP), without an Assurance to comply with the requirements of 45 CFR 46 to protect human research subjects. The restriction against using Federal funds for this purpose applies to all performance sites or suppliers of identifiable data, whether domestic or foreign, that does not have an OHRP-approved assurance. The awardee is responsible for determining whether or not performance sites are 'engaged' in human subject's research as defined by OHRP and must ensure the compliance of all performance sites. Refer to the OHRP website at <http://ohrp.osophs.dhhs.gov/> for details and guidance.

By accepting this award the grantee certifies that 1) its IRB has addressed the specific issue of "engagement" in human subjects research by its own organization as well as any other performance sites; 2) any performance site determined to be "engaged" in human subjects research will have an OHRP-approved Assurance in place, or be explicitly listed as an affiliated institution on another institution's Assurance document, before it performs any human subjects research; 3) the grantee will provide FDA with the name and address of each performance site requiring an assurance, and notify FDA if additional sites requiring assurances are identified post-award; 4) the grantee will provide FDA with the name and address of each performance site that the grantee's IRB has determined is not "engaged" in human subjects research; and 5) the grantee will provide FDA with Assurance numbers as they are approved for performance sites.

ALL institutions engaged in human subject research MUST file an 'assurance' of protection for human subjects with OHRP (45 CFR part 46). Applicants are advised to visit the OHRP Internet site at <http://www.hhs.gov/ohrp>. The requirement to file an assurance applies to both 'awardee', and collaborating 'performance site' institutions. No awardee or performance site may spend funds on human subject research or enroll subjects without the approved and applicable assurance(s) on file with OHRP.

NOTICE: ANIMAL USE IN RESEARCH: Under governing PHS Policy, Federal funds administered by the Public Health Service (PHS) shall not be expended for research involving live vertebrate animals without prior approval by the Office of Laboratory Animal Welfare (OLAW) of an Assurance to comply with the PHS Policy on Humane Care and Use of Laboratory Animals and the project has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC).

NOTE: See OLAW Resource-LINK indicating requirement on conducting activities on this project that may include animal use: <http://grants.nih.gov/grants/olaw/olaw.htm>

REPORTING REQUIREMENTS:

1. Quarterly program monitoring will be conducted which may be in the form of a telephone conversation between the Principal Investigator and the Project Officer/Grant Management Officer/Grants Management Specialist. Program monitoring may also be in the form of a site visit.
2. An annual Federal Financial Report (SF 425) is required. An original and one copy of this report must be submitted to the FDA Grants Management Officer within 90 days after the expiration date of the budget period.
3. An annual Program progress Report is required. The noncompeting continuation application will be considered the annual program progress report. The progress report must

include a description of the progress and accomplishments for each objective stated in the Request for Application as published in the Federal Register dated 08/25/2006.

4. All Non-Competing Continuation Applications (Type 5's) for future support are due to the Grants Management Specialist 3 months prior to the budget start date.

5. A Final Program Report, Federal Financial Report and Invention Statement must be submitted within 90 days after the expiration date of the project period.

The recipient will conduct, when appropriate, an annual Single Audit as required by OMB Circular A-133. This audit must be submitted to the Federal Audit Clearinghouse at the Bureau of the Census within 9 months of the close of their fiscal year.

If you need information on your organization's obligations under the Single Audit Act, please visit the following website: <http://harvester.census.gov/sac/> Valuable information is included under the "Frequently Asked Questions" section of that site.

All of the above must be mailed to the following address:

Vieda Hubbard, Grants Management Specialist
Food and Drug Administration
5630 Fishers Lane, RM 2034
Rockville, Maryland 20857
(240) 402-7588 (telephone)
(301) 827-0505 (fax)

SPECIAL TERMS AND CONDITIONS

DELINEATION OF SUBTANTIVE INVOLVEMENT

1. FDA will work closely with the grantee and have final approval on all project activities. This could include management structure for the program, development of plans and strategies for key scientific approaches and projects, and for identifying and carrying out the research.
2. FDA will participate in all functions directly related to the guidance and development of the program.
3. FDA will provide technical monitoring and/or direction of the work, including monitoring of data analysis, interpretation of analytical findings and their significance.
4. FDA will assist and approve (as deemed appropriate) the substance of publications, co-authorship of publications and data release.
5. FDA will have final approval on any re-directions proposed during the course of the project.
6. FDA will work closely with grantee on the Cosmetics botanical ingredients project. Funds cannot be expended until final protocols are approved by FDA.
7. FDA will work closely with grantee on a Workshop on Botanical cGMPs. The agenda will be developed with input from FDA.

FAILURE TO COMPLY WITH THE ABOVE STATE TERMS AND CONDITIONS COULD RESULT IN THE SUSPENSION OR TERMINATION OF THIS COOPERATIVE AGREEMENT.

All formal correspondence/reports regarding the grant should be signed by an authorized institutional official and the Principal Investigator and should be sent to the attention of the grants management specialist, unless otherwise directed.

For inquires regarding scientific programmatic issues and fiscal and/or administrative matters please refer to STAFF CONTACTS listed below:

Grants Management Specialist: Vieda Hubbard
Email: vieda.hubbard@fda.hhs.gov /Phone (240) 402-7588/ Fax (301) 827-0505

Program Official: Cara Welch
Email: cara.welch@fda.hhs.gov /Phone (240) 402-2333

Direct inquiries regarding scientific programmatic issues to the official listed below.

Direct inquiries regarding fiscal and/or administrative matters to the grants management specialist listed below.

All formal correspondence/reports regarding the grant should be signed by an authorized institutional official and the Principal Investigator and should be sent to the attention of the grants management specialist, unless otherwise explicitly directed.

STAFF CONTACTS

Grants Management Specialist: Gladys Melendez-bohler
Email: gladys.bohler@fda.hhs.gov Phone: 240-402-7565 Fax: (301) 827-0505

SPREADSHEET SUMMARY

GRANT NUMBER: 4U01FD004246-05

INSTITUTION: UNIVERSITY OF MISSISSIPPI

Budget	Year 5
Other Costs	\$1,341,590
TOTAL FEDERAL DC	\$1,341,590
TOTAL FEDERAL F&A	\$566,409
TOTAL COST	\$1,907,999

From: Pendleton, Kimberly
Sent: Wednesday, March 25, 2015 5:24 PM
To: ikhan@olemiss.edu
Cc: research@olemiss.edu; Welch, Cara; Geathers, LaQuia
Subject: Continuation Letter (U01FD004246-05) Ikhlas Ahmad Khan, Ph.D
Attachments: 2014Continuationletter.doc

Good Afternoon Dr. Khan,

Please find enclosed your FY2015 Continuation Letter which outlines submission instructions and funding level. If you should have any questions, please don't hesitate to contact me.

Best,
Kim
Kimberly Pendleton Chew, FDA
CGMO/Branch Chief

March 25, 2015

5U01FD004246-05

Ikhlas Ahmad Khan, Ph.D
120 Faser Hall, NCNPR
School of Pharmacy
University of Mississippi
University, MS 38677

Dear Dr. Khan,

All continuation applications (PHS2590 revised 06/09) are due prior to the start date of your award; therefore the continuation application for your FY2015 is due **06/01/2015**.

The anticipated total amount of available funds for the current budget period **09/01/2015 – 08/31/2016** is **\$2,437,442**. The amount includes direct and associated indirect costs as appropriate. **PLEASE NOTE:** All continuation funding is dependent upon the availability of funds, satisfactory progress, and timely submission of both progress and final reports.

The Non-Competing Grant Progress Report (PHS 2590) application form and instructions are available at <http://grants.nih.gov/grants/forms.htm>. To ensure your complete package submissions, please include the following forms:

Form Page 1 (Face page)

Form Page 2 (Detailed budget)

Form Page 3 (Budget Justification) – only if form page 2 is required

Form page 5 (Progress Report Summary) please include the following

Form page 6 (Checklist Page) – if there is a change in performance site(s) that will affect facilities and administrative costs and/or if program income is anticipated.

Form page 7 (Key Personnel Report)

Please submit your non-competing continuation application via e-mail to Kimberly.pendleton@fda.hhs.gov or hardcopy on or before June 1, 2015, to Kimberly Pendleton, 5630 Fishers Lane, Rockville, MD 20857, room 2031. Business inquiries may be directed to Kimberly Pendleton Chew, Chief Grants Management Officer/Branch Chief, (240) 402-7610. Programmatic inquiries may be directed to Cara Welch, PO at (240) 402-2333/cara.welch@fda.hhs.gov

Sincerely,

Kimberly Pendleton Chew

Kimberly Pendleton Chew, FDA
CGMO/Branch Chief
Office of Acquisitions & Grants Services

cc: Cara Welch, PO

From: Ikhlas Khan <ikhan@olemiss.edu>
Sent: Thursday, March 26, 2015 11:50 AM
To: Pendleton, Kimberly
Cc: Welch, Cara; Geathers, LaQuia
Subject: Re: Continuation Letter (U01FD004246-05) Ikhlas Ahmad Khan, Ph.D

Dear Kim
Thanks for the information. We will be submitting renewal shortly.
Thanks
IK

Ikhlas A. Khan, Ph.D, D. Litt (Hon. Causa)
Asst. Director, NCNPR
Director, FDA Center of Excellence
Director Center for Research in Indian Systems of Medicine (CRISM)
Director of Sino-US TCM Research Center
Research Professor Professor, Dept. of Pharmacognosy
National Center for Natural Products Research School of
Pharmacy University of Mississippi University, MS 38677
USA Tel 662/915/7821 fax 662/915/7989
<http://www.pharmacy.olemiss.edu/ncnpr/index.html>

From: <Pendleton>, Kimberly <Kimberly.Pendleton@fda.hhs.gov>
Date: Wednesday, March 25, 2015 at 4:23 PM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "research@olemiss.edu" <Research@olemiss.edu>, Cara Welch <Cara.Welch@fda.hhs.gov>, "Geathers, LaQuia" <LaQuia.Geathers@fda.hhs.gov>
Subject: Continuation Letter (U01FD004246-05) Ikhlas Ahmad Khan, Ph.D

Good Afternoon Dr. Khan,

Please find enclosed your FY2015 Continuation Letter which outlines submission instructions and funding level. If you should have any questions, please don't hesitate to contact me.

Best,
Kim
Kimberly Pendleton Chew, FDA
CGMO/Branch Chief

From: Ikhlas Khan <ikhan@olemiss.edu>
Sent: Thursday, August 28, 2014 12:13 PM
To: Hubbard, Vieda
Cc: Calvey, Elizabeth M; Geathers, LaQuia; Welch, Cara; research@olemiss.edu; Euphiazene Gray
Subject: Re: NoGA - U01 FD004246 Khan

Thanks so much.
Ik

Sent from my iPhone

On Aug 28, 2014, at 10:55 AM, "Hubbard, Vieda" <Vieda.Hubbard@fda.hhs.gov> wrote:

Grant Number – U01 FD004246
PI – Ikhlas A. Khan, Ph.D.

To All:

Please see the attached **Notice of Grant Award** of the above-mentioned. If you would like to have a hard copy of this award mailed to you, please notify me as this will be the only notification. If you do not require a hard copy, print the attached for your records.

Please Note: An **ANNUAL** Federal Financial Report (SF-425) is **REQUIRED**. A copy of this report must be submitted to the FDA Grants Management Office within 90 days after **each** budget period and within 90 days at the expiration date of the project period.

If I can further assist you, please contact me.

Vieda Hubbard
Grants Management Specialist
FDA/OAGS
vieda.hubbard@fda.hhs.gov
(240) 402-7588
(301) 827-0505 (fax)

<FE-FD4246-04 Khan.pdf>

From: Ikhlas Khan <ikhan@olemiss.edu>
Sent: Tuesday, September 03, 2013 1:25 PM
To: Hubbard, Vieda
Cc: Geathers, LaQuia
Subject: Re: NoGA - R01 FD004246 Khan

Thanks. We always happy to hear from you
Ik
Sent from my iPhone

On Sep 3, 2013, at 7:17 PM, "Hubbard, Vieda" <Vieda.Hubbard@fda.hhs.gov> wrote:

Grant Number – R01 FD004246
PI - Ikhlas A. Khan, Ph.D.

To All:

Please see the attached **Notice of Grant Award** of the above-mentioned. If you would like to have a hard copy of this grant mailed to you, please notify me as this will be the only notification. If you do not require a hard copy, print the attached for your records.

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If I can further assist you, please contact me.

Vieda Hubbard
Grants Management Specialist
FDA/OAGS
vieda.hubbard@fda.hhs.gov
(301) 827-7177
(301) 827-0505 (fax)

<FE-FD4246-03 Khan.pdf>

From: Ikhlas Khan <ikhan@olemiss.edu>
Sent: Tuesday, June 25, 2013 6:18 PM
To: Hubbard, Vieda
Cc: Geathers, LaQuia; Rader, Jeanne I *; research@olemiss.edu; TROY J SMILLIE
Subject: Re: Non-Competing Continuation Progress Report - U01 FD004246 (PI - Khan)

Dear MS Vieda

Thanks for the email and we will do our best to submit before 15th of July.

Thanks

Ik

From: "Hubbard, Vieda" <Vieda.Hubbard@fda.hhs.gov>
Date: Tue, 25 Jun 2013 21:49:21 +0000
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "Geathers, LaQuia" <LaQuia.Geathers@fda.hhs.gov>, "Rader, Jeanne I" <Jeanne.Rader@fda.hhs.gov>, "research@olemiss.edu" <Research@olemiss.edu>
Subject: Non-Competing Continuation Progress Report - U01 FD004246 (PI - Khan)

Dear Dr. Khan,

This email is being sent to inform you that there is funding for continuation of your cooperative agreement for the FY2013.

This information will also assist you in submitting your non-competing continuation progress report (PHS 2590 revised 08/12) under the University of Mississippi National Center for Natural Products Research (U01)(CFDA #93.103).

Funding for the referenced grant is scheduled to end **August 31, 2013**. In order to ensure consideration of future support, it will be necessary for your organization to complete and submit a Non-Competing Grant Progress Report (PHS 2590 revised 06/09) 60 days prior to the start of your next (to be funded) budget period to ensure timely continued support. **Please submit your non-competing continuation or request for no-cost extension no later than July 15, 2013.**

The required information for the non-competing grant progress report is similar to that requested by the FDA in the past with four exceptions, (1) you must respond to the questions on the Progress Report Summary Form Page 5 as detailed in the attachment to this letter; and (2) your report should not be submitted through Grants.gov.

Your Non-Competing Grant Progress Report shall be submitted to the FDA Grants Management Specialist noted at the end of this letter. All Reports shall be submitted as attachments to email or in hard copy through the mail.

The Non-Competing Grant Progress Report (PHS 2590) application form and instructions are available at <http://grants.nih.gov/grants/forms.htm> Please note that the instructions are oriented towards online submission and may include information which is not relevant to FDA. To ensure you submit a complete report, include the following application forms in your Progress Report:

- Form Page 1 (Face Page)

- Form Page 2 (Detailed Budget for Next Budget Period)
- Form Page 3 (Budget Justification)
- Form Page 5 (Progress Report Summary) - **Be sure to adequately address the questions on Form Page 5 in your Progress Report Summary as detailed in the attachment to this letter.**
- Submit a separate document providing a list of any changes to the protocol/research in the past year and provide an explanation for the changes.
- Form Page 7 (Key Personnel Report)
- Form Page 6 (Checklist Page) - if there is a change in performance site(s) that will affect facilities and administrative costs and/or if program income is anticipated.

If you have any questions, please contact the undersigned. Your completed Non-Competing Progress Report may be submitted as attachments via email or in hard copy to me as noted below.

If you are unable to submit your application by the cited due date, contact the undersigned immediately. Please note that funding is based on availability of funds and adequate progress on the grant. Should you have any questions, do not hesitate to contact me.

Thank you,

*Vieda Hubbard
Grants Management Specialist
FDA/OAGS/GAAT
(301) 827-7177
(301) 827-0505 (fax)*

From: Ikhlas Khan <ikhan@olemiss.edu>
Sent: Monday, November 07, 2011 3:23 PM
To: Melendez, Gladys; research@olemiss.edu
Cc: Kennedy, Barbara; Geathers, LaQuia; Brown, Tashea D
Subject: Re: 5 U01 FD002071-10 - Ikhlas Ahmad Khan - PI - NCNPR

Thank you so much.
IK

From: "Melendez, Gladys" <Gladys.Bohler@fda.hhs.gov>
Date: Mon, 7 Nov 2011 14:55:10 -0500
To: "'research@olemiss.edu'" <research@olemiss.edu>, Ikhlas Khan <ikhan@olemiss.edu>
Cc: "Kennedy, Barbara" <Barbara.Kennedy@fda.hhs.gov>, "Geathers, LaQuia" <LaQuia.Geathers@fda.hhs.gov>, "Brown, Tashea D" <Tashea.Brown@fda.hhs.gov>
Subject: FW: 5 U01 FD002071-10 - Ikhlas Ahmad Khan - PI - NCNPR

s a copy of a Notice of Grant Award reflecting a No Cost Extension and carryover on behalf of grant 5 U01 FD002071-10 - Ikhlas Ahmad Khan - PI - NCNPR
ords.

elendez
175

From: Geathers, LaQuia
Sent: Wednesday, August 02, 2017 2:29 PM
To: Fowler, Kiara
Cc: Ikhlas Khan; khan; Hannah Graham; Barbara Neyses; Amar Chittiboyina (amar@olemiss.edu); Swift, Sibyl; Welch, Cara; Brown, Tashea D
Subject: RE: Revised budget

Hi,

Its U01FD004246.

Thanks,
LaQuia

From: Fowler, Kiara
Sent: Wednesday, August 02, 2017 2:24 PM
To: Welch, Cara; Geathers, LaQuia; Brown, Tashea D
Cc: Ikhlas Khan; khan; Hannah Graham; Barbara Neyses; Amar Chittiboyina (amar@olemiss.edu); Swift, Sibyl
Subject: RE: Revised budget

Thank you Cara but I would need the full grant number.

Kiara

From: Welch, Cara
Sent: Wednesday, August 02, 2017 2:09 PM
To: Fowler, Kiara; Geathers, LaQuia; Brown, Tashea D
Cc: Ikhlas Khan; khan; Hannah Graham; Barbara Neyses; Amar Chittiboyina (amar@olemiss.edu); Swift, Sibyl
Subject: RE: Revised budget

UMiss Botanical Dietary Supplement Research grant (CFSAN-17-G-0947)

From: Fowler, Kiara
Sent: Wednesday, August 02, 2017 2:03 PM
To: Welch, Cara; Geathers, LaQuia; Brown, Tashea D
Cc: Ikhlas Khan; khan; Hannah Graham; Barbara Neyses; Amar Chittiboyina (amar@olemiss.edu); Swift, Sibyl
Subject: RE: Revised budget

Thank you,

Can you please provide me with the grant number?

Thank you,
Kiara

From: Welch, Cara
Sent: Wednesday, August 02, 2017 1:58 PM
To: Fowler, Kiara; Geathers, LaQuia; Brown, Tashea D

Cc: Ikhlas Khan; khan; Hannah Graham; Barbara Neyses; Amar Chittiboyina (amar@olemiss.edu); Swift, Sibyl
Subject: RE: Revised budget

Kiara, LaQuia, and Tashea,
As requested, UMiss has submitted a revised budget for \$2.058M.
Thank you
Cara

Cara Welch, Ph.D.
Senior Advisor
Office of Dietary Supplement Programs
CFSAN/FDA
Direct: 240-402-2333
Mobile: 301-452-5163
cara.welch@fda.hhs.gov

From: AMAR GOPAL CHITTIBOYINA [<mailto:amar@olemiss.edu>]
Sent: Wednesday, August 02, 2017 9:30 AM
To: Welch, Cara
Cc: Ikhlas Khan; khan; Hannah Graham; Barbara Neyses
Subject: Revised budget

Dear Dr. Welch,
As you suggested, the budget has been revised from \$2,158,000, to \$2,058,000 for Y2017 award. The statement of work and all other materials should remain the same for the cooperative agreement between NCNPR and ODSP/CFSAN. The excel file with couple of changes (highlighted in red) and justification are attached. Let us know if you need any additional information.
Thank you.

Sincerely,

Amar Chittiboyina, PhD
Assistant Director
NCNPR
The University of Mississippi
662-915-1572
amar@olemiss.edu

From: Fowler, Kiara
Sent: Tuesday, August 15, 2017 9:16 AM
To: AMAR GOPAL CHITTIBOYINA; Ikhlas Khan; research@olemiss.edu
Cc: AwardsCFSAN; Geathers, LaQuia; Brown, Tiffani; Brown, Tashea D; Welch, Cara
Subject: NOA
Attachments: NOA 4246.pdf

Importance: High

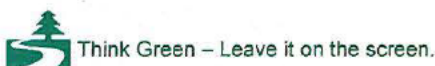
Good morning,

Attached is the Notice of Grant award, please let me know if you have any questions.

Thank you u

Kiara Fowler

Grants Management Specialist
Office of Operations, Office of Finance Budget & Acquisitions
Office of Acquisitions and Grants Services, HFA-500
U.S. Food and Drug Administration
Ph: 240-402-3099
Kiara.Fowler@fda.hhs.gov



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From: Fowler, Kiara
Sent: Thursday, January 12, 2017 7:48 AM
To: 'ccwhite@olemiss.edu'; 'research@olemiss.edu'; 'ikhan@olemiss.edu'; 'amar@olemiss.edu'
Cc: Geathers, LaQuia; Brown, Tashea D; Welch, Cara
Subject: Award 2U01FD004246-06 - Request to Change Co-PI
Attachments: 2U01FD004246.pdf

Good morning,

Attached is the new award for grant number 2U01FD004246-06 with the request to change co-pi.

Thank you

Kiara Fowler

Grants Management Specialist
Office of Operations, Office of Finance Budget & Acquisitions
Office of Acquisitions and Grants Services, HFA-500
U.S. Food and Drug Administration
Ph: 240-402-3099
Kiara.Fowler@fda.hhs.gov



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From: CHRISTY COX WHITE [<mailto:ccwhite@olemiss.edu>]
Sent: Wednesday, January 11, 2017 10:43 AM
To: Jones, Bryce
Cc: Ikhlas Khan; Sue Thorne
Subject: Award 2U01FD004246-06 - Request to Change Co-PI

Hi Mr. Jones,

The University of Mississippi respectfully requests to change the Co-PI on the above mentioned award. Dr. Larry Walker, current Co-PI, retired from UM effective 12/31/16.

We request that Dr. Amar Chittiboyina be named as Co-PI. Dr. Chittiboyina's CV is attached.

Please let me know if this acceptable or if additional information is needed. Thank you for your time.

Kindly,
Christy

Christy White, M.S.

Contracts and Grants Specialist

Office of Research and Sponsored Programs

The University of Mississippi

100 Barr Hall

University, MS 38677-1848

U.S.A.

+1-662-915-1710

ccwhite@olemiss.edu | www.olemiss.edu

From: Ikhlas Khan <ikhan@olemiss.edu>
Sent: Tuesday, September 03, 2013 1:25 PM
To: Hubbard, Vieda
Cc: Geathers, LaQuia
Subject: Re: NoGA - R01 FD004246 Khan

Thanks. We always happy to hear from you
Ik
Sent from my iPhone

On Sep 3, 2013, at 7:17 PM, "Hubbard, Vieda" <Vieda.Hubbard@fda.hhs.gov> wrote:

Grant Number – R01 FD004246
PI - Ikhlas A. Khan, Ph.D.

To All:

Please see the attached **Notice of Grant Award** of the above-mentioned. If you would like to have a hard copy of this grant mailed to you, please notify me as this will be the only notification. If you do not require a hard copy, print the attached for your records.

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If I can further assist you, please contact me.

Vieda Hubbard
Grants Management Specialist
FDA/OAGS
vieda.hubbard@fda.hhs.gov
(301) 827-7177
(301) 827-0505 (fax)

<FE-FD4246-03 Khan.pdf>

From: Brown, Tashea D
Sent: Wednesday, March 19, 2014 2:59 PM
To: Jennifer S. Taylor
Cc: Tien, Quyen; Ikhlas Khan; Geathers, LaQuia
Subject: RE: Updated SAM information! IMPORTANT

Good afternoon, I just checked SAM per your request and Dr. Khan is now listed. Thanks for your help.

Best

Tashea Brown
Acquisition Program Specialist
FDA.CFSAN.OM.ERT
(P) 240.402.2357
(F) 301.436.2629
Tashea.brown@fda.hhs.gov

From: Jennifer S. Taylor [mailto:jnnfrtyl@olemiss.edu]
Sent: Wednesday, March 19, 2014 1:52 PM
To: Tien, Quyen; Brown, Tashea D; Ikhlas Khan
Subject: RE: Updated SAM information! IMPORTANT

It seems that I didn't have something completed. I now have it complete. So please try again now.

Jennifer Taylor

Jennifer Taylor
Program Coordinator
University of Mississippi
National Center for Natural Products Research
301Q Thad Cochran
P.O. Box 1848
University, MS 38677

✉ jnnfrtyl@olemiss.edu
☎ 662-915-1090
📠 662-915-7989

From: Tien, Quyen [mailto:Quyen.Tien@fda.hhs.gov]
Sent: Wednesday, March 19, 2014 10:33 AM

To: Brown, Tashea D; Ikhlas Khan; Jennifer S. Taylor
Subject: RE: Updated SAM information! IMPORTANT
Importance: High

Tashea,

I'm copying Dr. Khan's assistant Jennifer who has helped Dr. Khan with the creation of the DUNS and registration in SAM.

Jennifer, please verify the DUNS number and SAM registration with us.

Thanks,
Quyen

From: Brown, Tashea D
Sent: Wednesday, March 19, 2014 11:29 AM
To: ikhlan@olemiss.edu
Cc: Tien, Quyen
Subject: Updated SAM information! IMPORTANT
Importance: High

Good morning Dr. Khan I have been tasked with processing your procurement package for the Office of Compliance for your expert witness services. As a part of the procurement process I have to verify that your information is current in SAM (System for Award Management). I entered the DUNS No: 079303650 and according to SAM this number is not there. Is it possible that it belongs to the University of Mississippi or it has expired? Can you please provide me with the correct information as soon as possible as this is a time sensitive action. **Contracts cannot be awarded to contractors who are not registered in SAM.**

Regards

Tashea Brown
Acquisition Program Specialist
FDA.CFSAN.OM.ERT
(P) 240.402.2357
(F) 301.436.2629
Tashea.brown@fda.hhs.gov

From: Calvey, Elizabeth M
Sent: Wednesday, April 30, 2014 12:26 PM
To: Geathers, LaQuia; Robinson, Kevin W.
Cc: Welch, Cara; Olson, Eric
Subject: FW: non-competitive continuation application

FYI.

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Wednesday, April 30, 2014 12:16 PM
To: Calvey, Elizabeth M; Larry Walker
Cc: Welch, Cara; Olson, Eric
Subject: Re: non-competitive continuation application

Dear Beth

I don't remember and can not find her email in that time frame about submission notice. We did exchange emails about grant # 3871 there were missing closing document in January. It was resolved.

We will keep the document ready as soon as we get her email.

Thanks
IK

From: calvey <elizabeth.calvey@fda.hhs.gov>
Date: Wed, 30 Apr 2014 15:41:02 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>, Larry <lwalker@olemiss.edu>
Cc: "Welch, Cara" <Cara.Welch@fda.hhs.gov>, Eric Olson <Eric.Olson@fda.hhs.gov>
Subject: non-competitive continuation application

Ikhlas and Larry,

I was just meeting with Scarlett and she showed me an e-mail that Gladys sent in late November to Rob Atwill the PI for the WCFS cooperative agreement regarding when to submit the non-competitive continuation application (90 days prior to the start of the new budget period). Can you please check your e-mail and see if you received one from Gladys in that time frame?

Beth

Elizabeth M. Calvey, Ph.D.
Director, Collaborative Partnerships
Senior Science Advisor Staff
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835
240-402-1981

From: Calvey, Elizabeth M
Sent: Thursday, October 31, 2013 10:30 AM
To: Geathers, LaQuia
Subject: RE: NoGA - R01 FD004246 Khan (U. MIss Award Copy - \$2.5 M)

thx

From: Geathers, LaQuia
Sent: Thursday, October 31, 2013 10:15 AM
To: Calvey, Elizabeth M
Subject: RE: NoGA - R01 FD004246 Khan (U. MIss Award Copy - \$2.5 M)

<< File: FE-FD4246-03 Khan.pdf >>

From: Calvey, Elizabeth M
Sent: Thursday, October 31, 2013 9:48 AM
To: Geathers, LaQuia
Subject: FW: NoGA - R01 FD004246 Khan (U. MIss Award Copy - \$2.5 M)

LaQuia,

Do you have a readable (openable) copy of the NOGA for UMiss?

Beth

From: Fabricant, Daniel
Sent: Thursday, October 31, 2013 9:46 AM
To: Calvey, Elizabeth M
Subject: RE: NoGA - R01 FD004246 Khan (U. MIss Award Copy - \$2.5 M)

Ask vieda for it I cant restore it

From: Calvey, Elizabeth M
Sent: Thursday, October 31, 2013 9:45 AM
To: Fabricant, Daniel
Subject: RE: NoGA - R01 FD004246 Khan (U. MIss Award Copy - \$2.5 M)

Can't open it, Can you restore the original document?

From: Fabricant, Daniel
Sent: Thursday, October 31, 2013 9:40 AM
To: Calvey, Elizabeth M
Subject: FW: NoGA - R01 FD004246 Khan (U. MIss Award Copy - \$2.5 M)

From: Hubbard, Vieda
Sent: Tuesday, September 03, 2013 1:17 PM
To: 'Ikhlas Khan'
Cc: Geathers, LaQuia; 'research@olemiss.edu'; Rader, Jeanne I; Trent, Brian
Subject: NoGA - R01 FD004246 Khan

Grant Number – R01 FD004246
PI - Ikhlas A. Khan, Ph.D.

To All:

Please see the attached **Notice of Grant Award** of the above-mentioned. If you would like to have a hard copy of this grant mailed to you, please notify me as this will be the only notification. If you do not require a hard copy, print the attached for your records.

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If I can further assist you, please contact me.

Vieda Hubbard
Grants Management Specialist
FDA/OAGS
vieda.hubbard@fda.hhs.gov
(301) 827-7177
(301) 827-0505 (fax)

<< File: FE-FD4246-03 Khan.pdf.html >>

From: Fowler, Kiara
Sent: Thursday, August 10, 2017 3:12 PM
To: 'JOY TATUM SHIDELER'
Cc: Geathers, LaQuia; Welch, Cara; Brown, Tiffani; Brown, Tashea D; AMAR GOPAL CHITTIBOYINA; Ikhlas Khan; research@olemiss.edu; Anita Randle; Nina Jones; Robin Buchannon
Subject: RE: SAM.Gov - UM Delinquent Federal Debt

Joy,

Thank you for notifying me this information. I believe this should not prevent you from any delays processing the grant funds.

Thank you,
Kiara Fowler

From: JOY TATUM SHIDELER [mailto:jtshidel@olemiss.edu]
Sent: Thursday, August 10, 2017 2:40 PM
To: Fowler, Kiara
Cc: Geathers, LaQuia; Welch, Cara; Brown, Tiffani; Brown, Tashea D; AMAR GOPAL CHITTIBOYINA; Ikhlas Khan; research@olemiss.edu; Anita Randle; Nina Jones; Robin Buchannon
Subject: FW: SAM.Gov - UM Delinquent Federal Debt
Importance: High

Kiara:

The University of Mississippi contacted two different agencies on Tuesday, August 8th, to resolve this matter. At this time, the University does not have any delinquent debt per discussion with Barbara with the Bureau of Fiscal Service. Barbara stated the debt case of \$59.08 was closed as of August 8th. She indicated it would take between 30 to 60 days to clear this flag in SAM.gov.

Prior to the SAM.gov notification, the University was not aware of any delinquent federal debt. Due to the small amount that Barbara said had cleared earlier this week, we can only assume it was a small clerical error. Is there any further action the University needs to take regarding this matter in order to prevent any delays in processing of grant funds?

Thank you,
Joy

Joy Shideler, CPA, CRA
Director of Accounting
The University of Mississippi
Office of Accounting
113 Falkner
P.O. Box 1848
University, MS 38677-1848
U.S.A.
O: +1-662-915-3436 | F: +1-662-915-7001
jtshidel@olemiss.edu | www.olemiss.edu

From: research@olemiss.edu
Sent: Thursday, August 10, 2017 1:05 PM
To: Anita Randle
Subject: Fwd: SAM.Gov

University of Mississippi
Office of Research and Sponsored Programs

(662) 915-7577 Fax
research@olemiss.edu

Begin forwarded message:

From: "Fowler, Kiara" <Kiara.Fowler@fda.hhs.gov>
Subject: [SAM.Gov](#)
Date: August 10, 2017 at 1:03:04 PM CDT
To: "Amar Chittiboyina (amar@olemiss.edu)" <amar@olemiss.edu>, Ikhlas Khan <ikhlan@olemiss.edu>, "research@olemiss.edu" <research@olemiss.edu>
Cc: "Geathers, LaQuia" <LaQuia.Geathers@fda.hhs.gov>, "Welch, Cara" <Cara.Welch@fda.hhs.gov>, "Brown, Tiffani" <Tiffani.Brown@fda.hhs.gov>, "Brown, Tashea D" <Tashea.Brown@fda.hhs.gov>

Good afternoon,

I am processing your year 7 grant funds that start on 09/01/2017, however it is showing a delinquent federal debt in [SAM.gov](#).

Thank you

Kiara Fowler

Grants Management Specialist
Office of Operations, Office of Finance Budget & Acquisitions
Office of Acquisitions and Grants Services, HFA-500
U.S. Food and Drug Administration
Ph: 240-402-3099
Kiara.Fowler@fda.hhs.gov



Think Green – Leave it on the screen.

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From: Hubbard, Vieda
Sent: Tuesday, September 03, 2013 1:32 PM
To: Ikhlas Khan
Cc: Geathers, LaQuia
Subject: RE: NoGA - R01 FD004246 Khan

Hi Dr. Khan,

You are always more than Welcome!

I hope all is well!

Vieda,

*Vieda Hubbard
Grants Management Specialist
FDA/OAGS/GAAT
(301) 827-7177
(301) 827-0505 (fax)*

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Tuesday, September 03, 2013 1:25 PM
To: Hubbard, Vieda
Cc: Geathers, LaQuia
Subject: Re: NoGA - R01 FD004246 Khan

Thanks. We always happy to hear from you
Ik
Sent from my iPhone

On Sep 3, 2013, at 7:17 PM, "Hubbard, Vieda" <Vieda.Hubbard@fda.hhs.gov> wrote:

Grant Number – R01 FD004246
PI - Ikhlas A. Khan, Ph.D.

To All:

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If I can further assist you, please contact me.

Vieda Hubbard
Grants Management Specialist
FDA/OAGS
vieda.hubbard@fda.hhs.gov
(301) 827-7177
(301) 827-0505 (fax)

<FE-FD4246-03 Khan.pdf>

From: JOY TATUM SHIDELER <jtshidel@olemiss.edu>
Sent: Thursday, August 10, 2017 2:40 PM
To: Fowler, Kiara
Cc: Geathers, LaQuia; Welch, Cara; Brown, Tiffani; Brown, Tashea D; AMAR GOPAL CHITTIBOYINA; Ikhlas Khan; research@olemiss.edu; Anita Randle; Nina Jones; Robin Buchannon
Subject: FW: SAM.Gov - UM Delinquent Federal Debt
Importance: High

Kiara:

The University of Mississippi contacted two different agencies on Tuesday, August 8th, to resolve this matter. At this time, the University does not have any delinquent debt per discussion with Barbara with the Bureau of Fiscal Service. Barbara stated the debt case of \$59.08 was closed as of August 8th. She indicated it would take between 30 to 60 days to clear this flag in SAM.gov.

Prior to the SAM.gov notification, the University was not aware of any delinquent federal debt. Due to the small amount that Barbara said had cleared earlier this week, we can only assume it was a small clerical error. Is there any further action the University needs to take regarding this matter in order to prevent any delays in processing of grant funds?

Thank you,
Joy

Joy Shideler, CPA, CRA
Director of Accounting
The University of Mississippi
Office of Accounting
113 Falkner
P.O. Box 1848
University, MS 38677-1848
U.S.A.
O: +1-662-915-3436 | F: +1-662-915-7001
jtshidel@olemiss.edu | www.olemiss.edu

From: research@olemiss.edu
Sent: Thursday, August 10, 2017 1:05 PM
To: Anita Randle
Subject: Fwd: SAM.Gov

*University of Mississippi
Office of Research and Sponsored Programs*

(662) 915-7577 Fax
research@olemiss.edu

Begin forwarded message:

From: "Fowler, Kiara" <Kiara.Fowler@fda.hhs.gov>

Subject: [SAM.Gov](#)

Date: August 10, 2017 at 1:03:04 PM CDT

To: "Amar Chittiboyina (amar@olemiss.edu)" <amar@olemiss.edu>, Ikhlas Khan <ikhlan@olemiss.edu>, "'research@olemiss.edu'" <research@olemiss.edu>

Cc: "Geathers, LaQuia" <LaQuia.Geathers@fda.hhs.gov>, "Welch, Cara" <Cara.Welch@fda.hhs.gov>, "Brown, Tiffani" <Tiffani.Brown@fda.hhs.gov>, "Brown, Tashea D" <Tashea.Brown@fda.hhs.gov>

Good afternoon,

I am processing your year 7 grant funds that start on 09/01/2017, however it is showing a delinquent federal debt in [SAM.gov](#).

Thank you

Kiara Fowler

Grants Management Specialist
Office of Operations, Office of Finance Budget & Acquisitions
Office of Acquisitions and Grants Services, HFA-500
U.S. Food and Drug Administration
Ph: 240-402-3099
Kiara.Fowler@fda.hhs.gov



Think Green – Leave it on the screen.

The information contained in this message may be privileged, confidential, and/or protected from disclosure by the U.S. Food & Drug Administration. If the reader of this message is not the intended recipient, or an employee or agent responsible for delivering this message to the intended recipient, you are hereby notified that any dissemination, distribution or copying of this communication is strictly prohibited. If you have received this communication in error, please notify the sender immediately by replying to the message and deleting it from your computer. Thank you...

From: Welch, Cara
Sent: Wednesday, August 02, 2017 1:58 PM
To: Fowler, Kiara; Geathers, LaQuia; Brown, Tashea D
Cc: Ikhlas Khan; khan; Hannah Graham; Barbara Neyses; Amar Chittiboyina (amar@olemiss.edu); Swift, Sibyl
Subject: RE: Revised budget
Attachments: Revised Budget_2017-2018_08022017.xlsx; Revised Budget Justification-2017-2018_08022017.docx

Kiara, LaQuia, and Tashea,
As requested, UMiss has submitted a revised budget for \$2.058M.
Thank you
Cara

Cara Welch, Ph.D.
Senior Advisor
Office of Dietary Supplement Programs
CFSAN/FDA
Direct: 240-402-2333
Mobile: 301-452-5163
cara.welch@fda.hhs.gov

From: AMAR GOPAL CHITTIBOYINA [mailto:amar@olemiss.edu]
Sent: Wednesday, August 02, 2017 9:30 AM
To: Welch, Cara
Cc: Ikhlas Khan; khan; Hannah Graham; Barbara Neyses
Subject: Revised budget

Dear Dr. Welch,
As you suggested, the budget has been revised from \$2,158,000, to \$2,058,000 for Y2017 award. The statement of work and all other materials should remain the same for the cooperative agreement between NCNPR and ODSP/CFSAN. The excel file with couple of changes (highlighted in red) and justification are attached. Let us know if you need any additional information.
Thank you.

Sincerely,

Amar Chittiboyina, PhD
Assistant Director
NCNPR
The University of Mississippi
662-915-1572
amar@olemiss.edu

Program Director/Principal Investigator (Last, First, Middle): Khan, Ikhlas, A.
 Sponsor: FDA U01 non-competitive renewal (RFA-FD-16-014)
 Project Title: Science based authentication, validation and safety of botanical ingredients
 Project Period: 9/1/2017 - 8/31/2018

Faculty Fringe Rate 32.31%
 Grad Asst Fringe Rate 8.00%
 Hourly Fringe Rate 3.00%

Personnel	Calendar Months	Revised Budget, FY18	FY18 Base Salary	Requested Salary	Requested Fringe Benefits	Year 7 Request
PI Ikhlas Khan	4.80	40.00%	\$	(b) (6)	(6)	
Co-I Amar Chittiboyina	6.00	50.00%	\$			
Sr. Research Scientist Shabana Khan	3.00	25.00%	\$			
Sr. Research Scientist Bharthi Avula	3.60	30.00%	\$			
Sr. Research Scientist Yan Hong Wang	1.80	15.00%	\$			
Res. Scientist Natascha Techen	9.00	75.00%	\$			
Res. Scientist Zulfiqar Ali	9.00	75.00%	\$			
Sr. Research Scientist Ahmed Osman	9.60	80.00%	\$			
Research Scientist Jianping Zhao	7.20	60.00%	\$			
Res. Scientist Vijayasankar Raman	10.20	85.00%	\$			
Res. Scientist Mei Wang	5.40	45.00%	\$			
Res. Scientist Cristina Avonto	9.00	75.00%	\$			
Post Doc Ji-Yeong Bae (50% FTE)	4.50	75.00%	\$			
Post Doc Iffat Parveen	9.00	75.00%	\$			
Post Doc Saqlain Haider	5.40	45.00%	\$			
Post Doc Vikas Kumar	7.20	60.00%	\$			
R&D Botanist Lal Jayaratna	10.20	85.00%	\$			
Web Developer Steven Hopper	10.80	90.00%	\$			
Project Coordinator **Jennifer Taylor	10.80	90.00%	\$			
Hourly Wages	8.00	100%	\$			
Graduate Students (2)	24.00	100%	\$			
Total Salaries and FB					\$	935,341
equipment					\$	158,190
supplies					\$	142,939
travel					\$	48,000
contractual services					\$	140,000
MOBOT subcontract					\$	59,987
Tuition \$8,190 per student, per year (2)					\$	16,380
Total Direct Costs					\$	1,500,837
F&A base					\$	1,266,280
F&A 44%				Target:	\$	557,163
Total Request				\$ 2,058,000	\$	2,058,000
Funds left to allocate				\$	(0)	

*Normally F&A positions that need a "Normally F&A Justification Form" completed, and a detailed explanation in the Budget Justification
 \$187,000 is the current NIH/FDA salary cap and does not use escalation from year to year

BUDGET JUSTIFICATION

A. PERSONNEL: \$935,341

Faculty and Professional Staff

PI, Dr. Ikhlas A. Khan, Director and Research Professor, National Center for Natural Products Research, Principal Investigator will devote 40% (4.8 calendar months) of his time to this co-operative agreement. Dr. Khan will be the contact point with NCNPR for interactions with FDA scientists. Dr. Khan has extensive research project administrative experience, has served as PI of several projects. Dr. Khan is currently working as a program director of FDA program at the Center. Dr. Khan will organize and coordinate the Steering Committee operation, and work closely with FDA officials and University administration to ensure administrative compliance and performance.

Co-PI, Dr. Amar G. Chittiboyina, Assistant Director, NCNPR – 50% (6.0 calendar months) effort. Dr. Chittiboyina will be responsible for all aspects of data management for the project. Dr. Chittiboyina will coordinate particularly with the botanists, geneticist, isolation, analytical chemistry investigators and biologists, as well as with FDA scientists involved in the project, to develop and modify the data management workflow. Additionally, Dr. Chittiboyina is on the organizing committee for the Annual Oxford International Conference on the Science of Botanicals (www.oxfordICSB.org). He works directly with Dr. Khan on a daily basis for scientific direction of major portions of NCNPR research efforts

Principal Scientist – (Dr. Shabana I. Khan) - 25% (3.0 calendar months) effort. Dr. Khan will be responsible for direction of the efforts at UM to evaluate the ADME, herb-drug, toxicological parameters for the natural products and botanical extracts. Dr. Khan's training is in biochemistry, and she has worked for many years in the screening development with natural products.

Senior Research Scientist, Analytical Chemist – (Dr. Bharathi Avula) – 30% (3.6 calendar months) effort. Research Scientist, National Center for Natural Products Research will be responsible for analytical method development. Dr. Avula is a trained pharmacist and analytical chemist. She has developed expertise over the years in analysis of botanicals. She will also be supervising Dr.'s Yan Hong Wang and Mei Wang in developing relevant analytical methods.

Senior Research Scientist, Analytical Chemist – (Dr. Yan Hong Wang) – 15% (1.8 calendar months) effort. Dr. Wang will be responsible for analytical method development. Dr. Wang is a natural products chemist with analytical background. He has developed an extensive expertise over the years in the analysis of botanicals.

Research Scientist, Plant Genetics – (Dr. Natascha Tehen) – 75% (9.0 calendar months) effort. Dr. Tehen will be responsible for plant genetics approaches applied to the authentication of botanical dietary supplements. She is experienced in all the molecular techniques needed to accomplish this task.

Research Scientist, Isolation Chemist – (Dr. Zulfiqar Ali) – 75% (9.0 calendar months) effort. Dr. Ali will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Sr. Research Scientist, Chemist – (Ahmad Osman) 80% (9.6 calendar months) effort. This individual will be responsible for isolating and identifying compounds of interest from various botanical sources. Additionally, this person will be instrumental in compiling scientific information with regard to identification techniques (analytical, NMR, etc.) for the authentication of botanical dietary supplements for the purpose of populating a dynamic web portal to assist FDA researchers and other stakeholders.

Research Scientist, Isolation Chemist – (Dr. Jianping Zhao) – 60% (7.2 calendar months) effort. Dr. Zhao's responsibility will be to prepare extracts of selected botanicals, develop NMR identification/authentication techniques, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards. Dr. Zhao has several years of experience in developing

analytical HPTLC techniques for the purpose of analysis and quantitation of various botanical ingredients in dietary supplements.

Research Scientist, Botanist – (Dr. Vijayasankar Raman) – 85% (10.2 calendar months) effort. Dr. Raman is responsible for living collection in medicinal plant garden and he is also an expert in taxonomy. He has setup a network of botanical institutes to coordinate collection effort and germplasm/ seed bank collections which is required for collection, identification and authentication of medicinal plants of interest, including wild collections and cultivated specimens. Dr. Raman will be working on macro and microscopy of botanicals, needed for species authentication. Microscopy provides an understanding of anatomical features of a plant, which is instrumental in differentiating the species and also in determining potential adulterants.

Research Scientist, Analytical Chemist – (Dr. Mei Wang) – 45% (5.4 calendar months) effort. Dr. Wang will develop analytical methods for the purpose of identifying botanical dietary supplement samples. Dr Wang has several years of experience in developing analytical GC techniques for the purpose of analysis and quantitation.

Research Scientist, Chemist – (Dr. Cristina Avonto) – 75% (9.0 calendar months) effort. Dr. Avonto will be responsible for the development of non-animal alternative methods for skin sensitization and to evaluate the sensitization potential of botanical ingredients in cosmetics and personal care products using *in chemico* and *in vitro* assays.

Post-Doctoral Research Associate, Isolation and Analytical Chemist – (Dr. Ji-Yeong Bae) – 75% (4.5 calendar months) effort. Dr. Bae will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards. Assist Dr. Avula on method development and method validation for botanicals.

Post-Doctoral Research Associate, Plant Genetics – (Dr. Iffat Parveen) – 75% (9.0 calendar months) effort. Dr. Parveen will be responsible for plant genetics approaches applied to the authentication of botanical dietary supplements. She will assist and train under Dr. Tehen for the molecular techniques needed to accomplish the proposed work.

Post-Doctoral Research Associate, Synthetic Chemist – (Dr. Saqlain Haider) – 45% (5.4 calendar months) effort. Dr. Haider will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards. In addition to isolation work, he will be responsible for identification and synthesis of small molecules hepatotoxicity.

Post-Doctoral Research Associate, Biology – (Dr. Vikas Kumar) – 60% (7.2 calendar months) effort. Dr. Kumar will be responsible for the development of *in-vitro* assays to assess the safety of botanical ingredients used in supplements, cosmetics and other personal care products.

R&D Botanist – (Lal Jayaratna) – 85% (10.2 calendar months) effort. This individual is responsible for maintaining the living collection of the centers medicinal plant garden. Additionally, this individual prepares voucher specimens, maintains the programs seed bank and provides assistance/tours for training sessions and workshops.

Web Developer – (Steven Hopper) 90% (10.8 calendar months) effort. This individual will be responsible for the development of a botanical dietary supplement information portal for the dissemination of relevant scientific data and FDA Dietary Supplement cGMP information. In addition to this he will be aiding in the data labeling, collection/reporting efforts for this project.

Technical and Administrative Staff

Program Coordinator (Jennifer Taylor) - 90% (10.8 calendar months) effort. Ms. Taylor is responsible to the PIs, to allow for adequate follow-up with reports, publications, file documentation, sample tracking, etc. Ms. Taylor also instrumental in vital logistical support for workshops, training sessions and conferences.

NOTE: The position of Program Coordinator is normally not allowed as direct costs under OMB circular A-21. However, we are requesting these direct costs be allowed due to the large scope of the project and the number of personnel to be managed and supported. This position is easily allocable to the project, and is reasonable given the size and nature of the project.

Hourly Wages – Hourly wage support will be utilized for temporary and student workers employed in support of the project. These may be utilized in support of administrative or research activities, ranging from filing and data entry to field/greenhouse or laboratory assistance.

Graduate Students (2) – The graduate students will receive training in natural products chemistry, analytical chemistry. These students will be enrolled in the Ph.D. program in the Department of Pharmacognosy, or one of the other academic Departments in the school of Pharmacy.

Fringe Benefits

Fringe benefits for faculty and staff are calculated at the University's average rate of 32.31% of salary. Fringe benefits for graduate research assistants are calculated at the University's average rate of 8.0% of stipend. Fringe benefits for students paid hourly (graduate or undergraduate) are calculated at the University's average rate of 3.0% of wages. The University uses average rates for budgeting fringe benefits; however, charges made to the sponsor will be for actual fringe benefits paid per individual.

B. CONSULTANT COSTS: None

C. EQUIPMENT: \$158,190

These funds are to support the capacity for analytical work that is required for the success of the program. These funds will be utilized to either purchase new complete systems or to upgrade other existing equipment HPLC, GC, CE or MS.

D. SUPPLIES: \$ 142,939

Primary commodity expenditures for the project will be for:

HPLC columns \$18,000

NMR/MS supplies (tubes, gases, columns) \$9,000

Chemicals, Reagents and Standards \$13,000

Microscopic supplies (slides, stains, storage cassettes) \$9,000

Chromatographic supplies (TLC plates, chromatographic media, solvents) \$42,939

Mol. Biology supplies \$14,000

Botanical collection/storage materials \$10,000

Garden/greenhouse tools/supplies \$ 11,000

Books, databases other reference materials \$8,000

Computer supplies \$8,000**

****These costs are for essential computer supplies which are devoted solely to the FDA project, and not for general use.

E. TRAVEL: \$48,000

Included in this category are:

Travel for FDA Dietary Supplement GMP training sessions.

Establishing collection arrangements and collaborations (National and International)

Travel to/from Washington or other COE's for meetings with FDA officials

Travel costs related to hosting workshop/conference (National and International)

F. CONTRACTUAL SERVICES: \$140,000

Estimated expenditures in this category include contractual/professional services for:

Collection & documentation of plant material \$8,000

Scale-up extraction/isolation \$ 9,000

Taxonomic verifications \$8,000

Maintenance contracts/repairs for analytical equipment \$45,000

Software/upgrades for analytical equip. \$12,000

Shipping, mailing costs \$4,000

Sub-Total: \$86,000

Estimated expenses for hosting conference:

Printing/PR \$8,000

Speaker reimbursements (20 @ \$1,500) \$30,000

Dinners/breaks \$ 10,000

Staffing \$ 6,000

Sub Total: \$54,000

G. SUBCONTRACT: \$ 59,987

A subcontract with Missouri Botanical Garden will be in place for the amount of \$ 59,987. Missouri Botanical Garden will be collecting the specimens for this project and will be providing expertise on identification of the specimens. Dr. Rainer Bussmann, the director and curator of the William L. Brown Center for Plant Genetic Resource (WLBC), will be the Missouri Botanical Garden representative working on the project.

H. INDIRECT CHARGES: \$ 557,163

Indirect costs are calculated in accordance with The University of Mississippi's rate agreement with DHHS, dated September 12, 2011. Indirect costs for research are calculated at 44.0% of Total Direct Costs less equipment, tuition remission, and the portion of each sub-grant or subcontract in excess of \$25,000.

From: [Ikhlas Khan](#)
To: [Hansen, Patricia A](#)
Subject: Re: ICSB
Date: Tuesday, March 24, 2015 7:23:08 PM

Thanks, give me your direct number or call me at 662 915 7821.

Call 3:00 PM EST

IK

From: <Hansen>, Patricia A <Patricia.Hansen@fda.hhs.gov>
Date: Tuesday, March 24, 2015 at 4:02 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: ICSB

Thursday, at 3:00 p.m. EST would work better for me. (Actually, anytime between 2:30 and 4:00 p.m. EST.) Just let me know.

PH

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Tuesday, March 24, 2015 4:40 PM
To: Hansen, Patricia A
Subject: Re: ICSB

Good that you can come. Hope you can find another speaker otherwise we do have good lineup. We can on Thursday

Thursday at 11:00 EST works for you? Or suggest any time you prefer except early in the morning, I do have a student committee to attend

IK

From: <Hansen>, Patricia A <Patricia.Hansen@fda.hhs.gov>
Date: Tuesday, March 24, 2015 at 2:30 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: ICSB

Ikhlas,

I was able to get the dates worked out and plan to travel to Oxford on Sunday, 4/12 and return to the Washington area on Tuesday, 4/14.

So far, I've not been able to secure a replacement speaker for Dr. Belsito. I have another call or two I can make, though, and would like to give that through the end of this week. If I don't have someone by then, we'll probably need to just go with who we have, unless you have other ideas.

A site visit just ahead of the conference will not work well for Dr. Katz's schedule. We would still like to plan for one, ideally this fiscal year. Perhaps you and I could start trading possible timeframes to see what might work. Would you have time for a call on Thursday?

PH

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Tuesday, March 24, 2015 11:32 AM
To: Hansen, Patricia A
Subject: Re: ICSB

Dear Pat

Hope you were bale to change the dates (b) (6) Please let me know about Site visit plan and your travel plan.

IK

From: <Hansen>, Patricia A <Patricia.Hansen@fda.hhs.gov>
Date: Wednesday, March 18, 2015 at 12:13 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: ICSB

Dialing now...

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Wednesday, March 18, 2015 1:07 PM
To: Hansen, Patricia A
Subject: Re: ICSB
Importance: High

Hi Pat

I called your number. I am in office please call me at 662 915 7821

Thanks

IK

From: <Hansen>, Patricia A <Patricia.Hansen@fda.hhs.gov>
Date: Wednesday, March 18, 2015 at 8:53 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: ICSB

Thanks for the quick reply, Ikhlas. Between 1 and 2 p.m. could probably work. My day tomorrow is more flexible, too, with morning being best. Let me know what you'd like to do.

PH

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Wednesday, March 18, 2015 9:45 AM
To: Hansen, Patricia A
Subject: Re: ICSB

Hi Pat

Thanks. You are right it will be better to talk on phone. Can I call you at 11:30 your time or between 1-2 P.M. Today we have FDA training here and I can call in between the sessions.

Tomorrow is more flexible. Let me know what works with you.

My tel# 662 915 7821

Cell# (b) (6)

IK

From: <Hansen>, Patricia A <Patricia.Hansen@fda.hhs.gov>
Date: Wednesday, March 18, 2015 at 7:34 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: ICSB

Good morning, Ikhlas.

I am sorry to hear that Don Belsito will not be able to participate after all. I will reach out to the head of CIR today and see if she would like to take Don's place or if there is someone else from the panel who might be available and interested. She will likely have questions about speaker travel expenses and registration. Shall I just send her in your direction or to someone on your staff?

Also, I may have a conflict now with the first day of the conference (4/13). For what day is the cosmetics session scheduled?

Last but not least, our director, Dr. Linda Katz, is interested in meeting you and the others involved in the work we are sponsoring and seeing the facilities and resources at NCNPR – a site visit, if you will. Could such a site visit be productively conducted at or close to the date of the conference or would it be better to schedule for a different time?

Please let me know your thoughts on all of this. If a phone call would be helpful, just let me know. Thanks.

Pat

(Sorry not to have replied sooner, but I have been out of the office a fair bit lately with some (b) (6) (b) (6) .)

Patricia A. Hansen, Ph.D.
Deputy Director
Office of Cosmetics and Colors, HFS-100
U.S. Food and Drug Administration
5100 Paint Branch Pkwy
College Park, MD 20740

Tel.: 240-402-1130

Fax: 301-436-2975

patricia.hansen@fda.hhs.gov

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Friday, March 06, 2015 1:21 PM

To: Hansen, Patricia A

Subject: ICSB

Importance: High

Dear Pat

Hope you are doing fine. Looking forward to meeting you here at Oxford. Agenda is being prepared. I just received an email from Donald Belsito that he can't come.

Would you like to add someone else in the session or its better to have little more time for each speaker.

Please let me know what you would like to do.

Diegos slot will be filled by our guys talking about in vitro screening

Here are the speakers in your session, which you will chair

SESSION 2a: "Safety and Quality of Cosmetic Products "

Moderator and Session Chair:

<!--[if !supportLists]--> <!--[endif]-->**Patricia Hansen**, Deputy Director, FDA, OFVM, CFSAN, OCC, *"Cosmetics - FDA Science And Regulatory Perspectives"*

<!--[if !supportLists]--> <!--[endif]-->**Kimberley Norman**, Director, Institute for In Vitro Sciences, Inc.

<!--[if !supportLists]--> <!--[endif]-->**Donna McMillan**, Research Fellow, Proctor & Gamble *"Safety Of Botanical/Natural Substances In Cosmetic Products – An Industry Perspective"*

<!--[if !supportLists]--> <!--[endif]-->**Cristina Avonto, Ph.D**, University of Mississippi, In-vitro screening for safety assessment

IK

From: [Ikhlas Khan](#)
To: [Welch, Cara](#); [Hansen, Patricia A](#); [Ghulam Nabi Qazi](#); [Roe, Amy](#); [rick](#); [Betz, Joseph M \(NIH\)](#); [Michael Smith](#); [Dean Guo](#); [Larry Walker](#); [Stefan Gafner](#); [Gurley Jr., Billy J](#); [XING CONG LI](#); [Mahmoud A. Elsohly](#); [Saldanha, Leila G. \(NIH\)](#)
Cc: [Jennifer S. Taylor](#)
Subject: Chair person
Date: Wednesday, April 01, 2015 5:06:45 PM
Attachments: [OXFORD 15th ANNUAL CONFERENCE 2015 PROGRAM 3-31_15\[3\].docx](#)

Dear All

Some of you know that your chairing a session and for some sessions I took the liberty to assign you as chair and hoping that you don't mind assisting us. Biosketches of presenters will be included in the booklet but if you need information about them or need biosketches, Jennifer will be happy to provide them to you. Looking forward to having excellent conference once again with all your cooperation. Agenda is attached.
IK.

DAY 1 (MONDAY, APRIL 13)

8:00 -8:45 Open onsite registration – Oxford Conference Center (OCC) Lobby

8:45-10:00 Opening Session - OCC Auditorium

Welcome and Preliminary Remarks

- **Ikhlas Khan**, Associate Director, National Center for Natural Products Research (NCNPR), University of Mississippi
- **David Allen**, Dean and Executive Director of the Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi

Special Guests – introduced by Larry Walker, Director, NCNPR

- **Steven Musser**, Deputy Director for Scientific Operations, US Food and Drug Administration
- **Sh. Nilanjan Sanyal**, Secretary, Ministry of AYUSH (Ayurveda, Unani, Siddha and Homeopathy), New Delhi, India

Welcome on behalf of the University and Introduction of Keynote Speaker

- **Alice Clark**, Vice Chancellor for Research and Sponsored Programs, University of Mississippi

KEYNOTE ADDRESS

- **Josephine Briggs**, Director, NIH and National Center for Complementary and Integrative Health (NCCIH) *“Evidence-Based Health Care And The Integration Of Complementary Therapies”*

10:00-10:30

Break

SESSION 1: *“Update and Future Perspectives from the Regulators”*

Moderator and Session Chair: Cara Welch, Acting Director, Division of Dietary Supplement Programs

10:30-12:00 **Cara Welch**, Acting Director of the Division of Dietary Supplement Programs, CFSAN, FDA, *“FDA Overview: GMP, NDI, and Other Regulatory Responsibilities”*
Saleh Turujman, Chemist, Dietary Supplement Regulations Implementation Team, DDSP, CFSAN, FDATBD, *“An FDA Overview: Industry Specifications”*
Steven Casper, Biologist, NDI Review Team, DDSP, CFSAN, FDA, *“Avoiding NDI Notification Pitfalls”*
Jason Humbert, Senior Regulatory Manager, HHS/FDA/ORA/OGROP/ORA/OO/OEIO/DE, *“Dietary Supplements” that Contain Hidden Drugs*

12:00-12:15

Conference Photograph

Meet at OCC side patio across from Cedar Room Dining Hall.

12:15-1:30

Lunch - OCC Cedar Room Dining Hall

SESSION 2a: *“Safety and Quality of Cosmetic Products”*

Moderator and Session Chair: Patricia Hansen, Deputy Director, FDA, OFVM, CFSAN, OCC

1:30-2:00 **Patricia Hansen**, Deputy Director, FDA, OFVM, CFSAN, OCC, *“Cosmetics - FDA Science and Regulatory Perspectives”*

2:00-2:30 **Kimberly Norman**, Director, Institute for In Vitro Sciences, Inc., *“Using Novel in vitro Methods to Predict the Skin Sensitization Potential of Botanicals”*

(Cont'd) DAY 1 (MONDAY, APRIL 13)

2:30 - 2:45

Break



15th Annual Oxford International Conference on the Science and Regulation of Botanicals

- 2:45-3:15 **Donna McMillan**, Research Fellow, Proctor & Gamble *"Safety of Botanical/Natural Substances in Cosmetic Products – An Industry Perspective"*
- 3:15-3:45 **Cristina Avonto**, University of Mississippi, *"In chemico screening for skin sensitization risk assessment"*

SESSION 2b: *"Natural Products Discovery and Development in China"*

Moderator and Session Chair: **Ghulam Nabi Qazi**, Vice Chancellor, Jamia Hamdard (Hamdard University)

- 1:30-2:00 **Ai-jun Hou**, Professor, Fudan University, *"The Moraceae Family: An Important Source of Drug Discovery and Development"*
- 2:00-2:30 **Yue-wei Guo**, Professor, Shanghai Institute of Materia Medica, CAS, *"Exploring for New Secondary Metabolites with Pharmacological Potential from the Mangroves of Southern Coast of China"*

2:30 - 2:45	Break
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- 2:45-3:15 **Yi-Zhun Zhu**, Professor, Fudan University, *"Discovery and development of SCM-198, a novel compound from Chinese Herb for ischemic stroke"*
- 3:15-3:45 **Yong Qin**, Professor, SiChuan University, *"Asymmetric Total Syntheses of Lundurine A and Isoschizogamine"*
- 3:45-4:15 **Xia-dong Luo**, Professor, Kunming Institute of Botany, CAS, *"Indole Alkaloids from Plants: Novel Structures, Bioactivities and Preclinical Investigation"*

SESSION 3: *"An update on the NY Attorney General's Office action on botanical dietary supplements: what is at stake and what the industry and scientific community are doing about this"*

Moderators: **Loren Israelsen** - President - United Natural Products Alliance; **Mark Blumenthal** - Executive Director - American Botanical Council; **Nandajumara Sarma** - Director / Dietary Supplements - US Pharmacopeia

4:15-5:45 Panel Discussion

6:00- 8:00	Reception/Mixer Oxford Conference Center Cedar Room Presentation of the Waters Award for Outstanding Contribution in Natural Product Science
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DAY 2 (TUESDAY, APRIL 14)



15th Annual Oxford International Conference on the Science and Regulation of Botanicals

SESSION 4: ***“NY: The necessity of establishing a new quality and safety paradigm for dietary supplements”***

Moderator and Session Chair: Rick Kingston, President, Regulatory/Scientific Affairs, Safety Call

8:30-10:00 **Rick Kingston**, President, Regulatory/Scientific Affairs, Safety Call International & Clinical Professor, University of Minnesota, Adjunct Professor University of Mississippi, *“A framework for instilling clinician confidence in botanical dietary supplements post NY AG action”*

Victor Navarro, Chairman, Division of Hepatology, Einstein Medical Center, *“Liver Injury Due To Herbal and Dietary Supplements”*

Pieter Cohen, Assistant Professor of Medicine, CHA Somerville Hospital Primary Care, *“Clinician’s Perspective On The Dietary Supplement Health And Education Act Of 1994’s (DSHEA) Ability To Ensure The Safety Of Dietary Supplements”*

10:00-10:30

Break

SESSION 4 CONTINUED: ***“NY: The necessity of establishing a new quality and safety paradigm for dietary supplements”***

Moderator and Session Chair: Rick Kingston, President, Regulatory/Scientific Affairs, Safety Call

10:30-12:00 **Jason Little**, Director of Dietary Supplement Toxicology, New Chapter Inc/Procter and Gamble, *“Dietary Supplement Safety Science and Industry Stewardship: A Corporate Perspective”*

Paul Jacobson, CEO, Thorne Research, *“Raising the Bar in Product Quality, Safety, and Efficacy”*

PANEL DISCUSSION Co-Moderator, **Brent Bauer**, Integrative Medicine Program, Mayo Clinic & **Michael O’Hara**, Global Nutraceuticals UL, LLC

12:00-1:00

Lunch - OCC Cedar Room Dining Hall

SESSION 5a: ***“Pre-market safety considerations of dietary supplements: importance of assessing potential Herb-Drug Interactions”***

Moderator and Session Chair: Amy Roe, Associate Director, Global Business Services, the Procter & Gamble Company

1:00-1:30 **Mary Paine**, Associate Professor, Washington State University, *“Natural Product-Drug Interactions: From Quantitative Prediction to Clinical Assessment”*

1:30-2:00 **Reginald Frye**, Professor, University of Florida, *“Potential for natural product-drug interactions mediated through conjugative drug metabolism”*

2:00 - 2:30

Break

(Cont’d) DAY 2 (TUESDAY, APRIL 14)



15th Annual Oxford International Conference on the Science and Regulation of Botanicals

- 2:30-3:00 **Shabana Khan**, Principal Scientist, University of Mississippi, *"Characterization of ADME Properties and Assessment of Drug Interaction Potential of Botanicals"*
- 3:00-3:30 **Bill Gurley**, Professor, University of Arkansas for Medical Science, *"Emerging Technologies for Improving Phytochemical Bioavailability: Benefits and Risks"*
- 3:30-4:00 **Jonathan Jackson**, Chief Executive Officer, Qualyst, *"In Vitro Evaluation of Schisandra Extracts (SE) Herb-Drug Interaction Potential Utilizing B-Clear Sandwich-Culture Human Hepatocytes (SCHH)"*

5:30-8:00 **Poster Session Chair:** Leila G Saldanha, PhD, RD, Scientific Consultant, Office of Dietary Supplements, National Institutes of Health

7:00 – 8:00 **Dinner - OCC Cedar Room Dining Hall**

(Cont'd) DAY 2 (TUESDAY, APRIL 14)

SESSION 5b: *"Quality Assessment and Analytical Techniques"*

Moderator and Session Chair: Joseph M. Betz, Director, Analytical Methods and Reference Materials Program Office of Dietary Supplements National Institutes of Health

- 1:00-1:30 **Joe Chang**, Chief Scientific Officer and Executive Vice President, Product Development, Nu Skin, *"Commercialization Of Medicinal Herbs: What's Good For The Goose Is Not Good For The Gander"*
- 1:30-2:00 **Brian Schaneberg**, Director of Regulatory & Scientific Affairs, Starbucks Coffee Company, *"From Tree to Cup, the Journey of A Coffee Bean"*

2:00 - 2:30 **Break**

- 2:30-3:00 **Corey Hilmas**, Senior Vice President of Scientific & Regulatory Affairs, Natural Products Association
- 3:00-3:30 **Giorgis Isaac**, Principal Scientist Pharmaceutical Business Operations, Waters Corporation, *"Natural Product Chemical Ingredient Profiling using Novel Analytical and Informatics Tools"*
- 3:30-4:00 **Jerry Zweigenbaum**, Senior Research Scientist, Agilent Technologies, *"Characterization of Pyrrolizidine Alkaloids in Botanicals and Dietary Supplements by LC/QTOF MS"*
- 4:00-4:30 **Jean-Luc Wolfender**, Professor, University of Geneva, *"New Ways to Decipher Plant and Microbial Metabolomes"*

5:30-8:00 **Poster Session Chair:** Leila G Saldanha, PhD, RD, Scientific Consultant, Office of Dietary Supplements, National Institutes of Health

7:00 – 8:00 **Dinner - OCC Cedar Room Dining Hall**

DAY 3 (WEDNESDAY, APRIL 15)



15th Annual Oxford International Conference on the Science and Regulation of Botanicals

SESSION 6: *"International Perspectives on Regulation of Botanicals"*

Moderator and Session Chair: Michael Smith, President, Michael J Smith & Associates, Adjunct Fellow, University of Western Sydney

- 8:30-9:00 **Bruce Randall**, Acting Director, Health Products Acting Director, Health Products & Food Branch, Health Canada, *"New Approach to Quality for Natural Health Products Regulated By Health Canada"*
- 9:00-9:30 **Werner Knöss**, Department Head, Federal Institute for Drugs and Medical Devices, *"European Regulatory Framework for Herbal Medicinal Products – Challenges For Traditional Medicines"*
- 9:30-10:00 **David Briggs**, Professor, University of Western Sydney, *"Issues in the Regulation of Herbal Medicines: An Australian Perspective"*

10:00-10:30	Break
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SESSION 7a: *"Botanical Development and Regulation"*

Moderator and Session Chair: Mahmoud ElSohly, Research Professor, National Center for Natural Products Research, University of Mississippi

- 10:30-11:00 **Olivier Rabin**, Science Director, World Anti-Doping Agency, *"Dietary Supplements and Sport: Stronger Regulation Needed!"*
- 11:00-11:30 **Charles Wu**, Pharmacologist, Botanical Review Team, Food and Drug Administration, *"Totality-of-Evidence Approach to Evaluation of Botanical Drug-US FDA New Botanical Guidances"*
- 11:30-12:00 **Steven Gust**, NIDA International Program Director, National Institute of Health, *"Research on Marijuana and Its Constituents: Recent Developments and Current Regulatory Environment"*

12:00-1:00	Lunch - OCC Cedar Room Dining Hall
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1:30-2:30	DEDICATION CEREMONY
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	Thad Cochran Research Center, West Wing Research Facility National Center for Natural Products Research
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3:00-9:00	ICSB Picnic - Oxford Conference Center. Food, Fun, Music and Games
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SESSION 7b: *"Asian Traditional Medicines"*

Moderator and Session Chair: De-an Guo, Professor, Shanghai Institute of Materia Medica, CAS

- 10:30-11:00 **Mian Zhang**, Professor, China Pharmaceutical University, *"Morphological and Chemical Identification on Tibetan Medicine Flos Asteris"*

(Cont'd) DAY 3 (WEDNESDAY, APRIL 15)



15th Annual Oxford International Conference on the Science and Regulation of Botanicals

- 11:00-11:30 **Wei Wang**, Professor, Hunan University of Chinese Medicine, *"Chemical, Pharmacological and Quality Control Studies of Traditional Chinese Medicine (TCM) and Ethnic Medicine"*
- 11:30-12:00 **Pulok Mukherjee**, Jadavpur University, *"Evidence-based Validation of Traditional medicine- Farm to Pharma"*

12:00-1:00 **Lunch - OCC Cedar Room Dining Hall**

1:30-2:30 **DEDICATION CEREMONY**
Thad Cochran Research Center, West Wing Research Facility
National Center for Natural Products Research

3:00-9:00 **ICSB Picnic - Oxford Conference Center. Food, Fun, Music and Games**

DAY 4, (THURSDAY, APRIL 16)

SESSION 8: "International Efforts on the Development of Traditional Medicines"

Moderator and Session Chair: **Larry Walker**, Director, National Center for Natural Products Research, University of Mississippi

- 8:30-9:00 **Chau Van Minh**, President of VAST, Vietnam Academy of Science and Technology, *"Biodiversity and Chemical Study of Plants and Marine Organisms in Vietnam"*
- 9:00-9:30 **Haruki Yamada**, Professor, Tokyo University of Pharmacy & Life Sciences, *"Elucidation of Action Mechanism of Multi-Ingredient Drugs, Kampo (Japanese Traditional) Medicines and Their Roles in Modern-Day Medicine"*
- 9:30-10:00 **Young Ho Kim**, Professor, Chungnam National University, *"Potential Anti-Osteoporotic and Antioxidant Components from Artemisia Iwayomogi and Prunus Mume"*

10:00-10:30 **Break**

SESSION 9: "Integrity and Quality of Botanicals"

Moderator and Session Chair: **Stefan Gafner**, Chief Science Officer, American Botanical Council

- 10:30-11:00 **Thomas Brendler**, Plantaphile, *"Natural Products Regulations in the European Union-An Update"*
- 11:00-11:30 **Josef Brinckmann**, Vice President of Sustainability, Traditional Medicinals, *"Concurrent Trends for Geographical Indication Herbs and Locally Grown Herbs: Are There Implications for Pharmacopoeial Quality or Therapeutic Efficacy?"*
- 11:30-12:00 **Mark Blumenthal**, Founder & Executive Director, American Botanical Council, *"Laboratory Guidance on Analytical Methods to Detect Adulterants in Commercial Botanical Materials: The Next Phase of the ABC-AHP-NCNPR Botanical Adulterants Program"*

12:00-1:00 **Lunch - OCC Cedar Room Dining Hall**

(Cont'd) DAY 4, (THURSDAY, APRIL 16)



15th Annual Oxford International Conference on the Science and Regulation of Botanicals

SESSION 10a: ***"Biological Aspects in the Characterization of Natural Products"***

Moderator and Session Chair: Bill Gurley, Professor, University of Arkansas for Medical Science

1:00-1:30 **De-a Guo**, Shanghai Institute of Materia Medica, CAS, *"Salvia Miltiorrhiza: From the Field to the Bedside"*

1:30-2:00 **Pang-Chui Shaw**, the Chinese University of Hong Kong, *"Identification of Herbal Components in Decoctions and Medicinal Granules by DNA Markers"*

2:00 - 2:30

Break

2:30-3:00 **James Traub**, Senior Business Development Manager in Natural Products, Waters Corporation, *"Mass Detection for the Masses: Benefits of UPLC/QDA for Routine Analysis of Dietary Supplements"*

3:00-3:30 **Irmgard Merfort**, Albert Ludwigs University of Freiburg, *"Arnica and Birch – Two Examples for Successful European Phytomedicines"*

Afternoon tour of NCNPR facilities or Medicinal Plant Garden

6:30

Closing Ceremony and Banquet (OCC Cedar Room)

Registration is required and is available online and onsite

SESSION 10b: ***"Discovery and Development of Novel Therapeutics from Natural Sources"***

Moderator and Session Chair: Xing-Cong Li, Principal Scientist, National Center for Natural Products Research, University of Mississippi

1:00-1:30 **Alexander Shikov**, St. Petersburg Institute of Pharmacy, *"Potential of Green Sea Urchins as a Source of Medicinal Preparations"*

1:30-2:00 **Esperanza Carcache de Blanco**, The Ohio State University, *"Bioassay Development and Natural Products"*

2:00 - 2:30

Break

2:30-3:00 **James D McChesney**, Arbor Therapeutics, LLC, *"Effective Healing Of Cutaneous Leishmaniasis Using Standardized Topical Preparation with Solanum Lycocarpum Fruit Glycoalkaloids"*

3:00-3:30 **Pratima Tatke**, C.U.Shah College of Pharmacy, SNDT Women's University, *"Wound Healing Activity Of Topical Gel Containing Methanol Extract Of Salvadora Persica Linn. Stem On Fresh Wound in Rats"*

Afternoon tour of NCNPR facilities or Medicinal Plant Garden

6:30

Closing Ceremony and Banquet (OCC Cedar Room)

Registration is required and is available online and onsite

From: [Ikhlas Khan](#)
To: [Hansen, Patricia A](#)
Subject: Re: Thanks
Date: Thursday, May 21, 2015 4:17:20 PM

Sure
ik

From: <Hansen>, "patricia.hansen@fda.hhs.gov" <patricia.hansen@fda.hhs.gov>
Date: Thursday, May 21, 2015 at 3:04 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Thanks

Ikhlas – I am running a little behind, with meetings back-to-back all day. Can I call you a little later, say at 5:00?
PH

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Thursday, May 21, 2015 1:00 PM
To: Hansen, Patricia A
Subject: Re: Thanks

Good
ik

From: <Hansen>, "patricia.hansen@fda.hhs.gov" <patricia.hansen@fda.hhs.gov>
Date: Thursday, May 21, 2015 at 11:54 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Thanks

I will call you!
Thanks.
PH

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Thursday, May 21, 2015 12:53 PM
To: Hansen, Patricia A
Subject: Re: Thanks

Fine, what number should I call.
My Office# 662 915 7821
IK

From: <Hansen>, "patricia.hansen@fda.hhs.gov" <patricia.hansen@fda.hhs.gov>
Date: Thursday, May 21, 2015 at 11:50 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Thanks

Hi, Ikhlas. Best time block for me today will be after 4:30. Does that work for you?

PH

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Sunday, May 17, 2015 5:21 PM

To: Hansen, Patricia A

Subject: Re: Thanks

Hi Pat

It will be nice if we can talk on Thursday so I know what to prepare. I just realized Monday is a holiday

IK

From: <Hansen>, "patricia.hansen@fda.hhs.gov" <patricia.hansen@fda.hhs.gov>

Date: Sunday, May 17, 2015 at 3:58 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Subject: RE: Thanks

Ikhlas,

This looks just right. Thanks.

FYI – I will be out for the next couple of days. Let me know if there is anything else we should discuss ahead of time and I will find some time on Thursday/Friday for a call.

PH

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Wednesday, May 13, 2015 2:29 PM

To: Hansen, Patricia A

Cc: Russell, Sandra

Subject: Re: Thanks

Hi Pat

Thanks for confirming the time. Jennifer will get in touch with Sandy shortly.

I am just thinking as follows.

Arrival at 11:00

Introduction to group

Overview of the on going program

Lunch

Tour of the facility

Discussion

Tour of the garden

Checkin for hotel

Dinner together

I am open for any suggestion

IK

From: <Hansen>, "patricia.hansen@fda.hhs.gov" <patricia.hansen@fda.hhs.gov>

Date: Wednesday, May 13, 2015 at 1:02 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Russell, Sandra" <Sandra.Russell@fda.hhs.gov>

Subject: RE: Thanks

Ikhlas,

I'm glad to hear it. We will arrive in Memphis a few minutes after 9 a.m., and so I think we should be arriving in Oxford sometime between 10:30 and 11:00 a.m. We will be renting a car and so will need directions, parking information, etc. If you could have someone send that to Linda's and my administrative assistant, Sandy Russell (cc'd on this note), she will make sure to get that into our travel folders. If you or your staff need more information about our travel arrangements, she will be able to provide it.

Pat

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Tuesday, May 12, 2015 4:50 PM

To: Hansen, Patricia A

Subject: Re: Thanks

Hi Pat

Yes, its fine, we can spend the rest of the day on 26th for discussion and give a tour of the building and botanical garden

You can leave on 27th morning.

Please let me know when you are planning to come so we can arrange accordingly

IK

From: [Ikhlas Khan](#)
To: [Hansen, Patricia A](#)
Subject: Thanks
Date: Friday, May 29, 2015 2:57:32 PM

Dear Pat

Would like to thank for arranging the visit, I hope it was worth the trip. We certainly felt more comfortable and got an idea what you are looking for.

You will be getting busy with your new duties but hope you will continue to advise us on both sides.

Thanks

IK

From: [Ikhlas Khan](#)
To: [Katz, Linda](#)
Cc: [Sadrieh, Nakissa](#); [Milstein, Stanley R](#); [AMAR GOPAL CHITTIBOYINA](#); [CRISTINA AVONTO](#)
Subject: Re: list
Date: Friday, July 10, 2015 11:16:03 AM
Attachments: [inchemico methods case studies.pptx](#)
[FDA summary in vitro assays-v2.pptx](#)
[ARBUTIN summary.pptx](#)
[Arbutin stability.pdf](#)
[14123 Wang_Y.pdf](#)

Dear Linda

Attached, please find separate ppts to describe the results and approaches. Some slides might need further explanation, we can setup a call to go through over these slides if needed.

In the meantime anyone has a question, please feel free to contact us.

Have a nice weekend

IK

And publications

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Monday, June 29, 2015 at 12:45 PM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>
Subject: RE: list

Thanks very much!

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Monday, June 29, 2015 1:04 PM
To: Katz, Linda
Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: list

Dear Linda

We will send the information soon.

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Monday, June 29, 2015 at 10:48 AM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>
Subject: RE: list

Ikhlas,

As you know there have been a number of personnel changes in OCAC. In order for us to

plan our next steps and discuss further research needs including and beyond the allergens, it would be helpful for all of those involved in OCAC to be on the same page.

Please provide a summary of the in vitro sensitization assays available and the ingredients tested at your facility as well as the methods that will be used to assess the allergens of current concern. In addition, it would be helpful if you could provide us with a summary of the work done on arbutins, focusing on the scientific questions asked, methods developed, experiments performed and the results/conclusions. Nakissa will be working on our collaborative research projects for the interim and she may have additional questions for you. Thanks again for your help. (Your response needs not be extensive.)

Linda

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Monday, June 29, 2015 10:55 AM
To: Katz, Linda
Cc: Hansen, Patricia A; Sadrieh, Nakissa; Milstein, Stanley R
Subject: Re: list

Dear Linda

Thanks for sharing the list and the related information. We will collect information about the listed compounds and will be ready to discuss next steps soon.

Thanks

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Thursday, June 25, 2015 at 9:47 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: "patricia.hansen@fda.hhs.gov" <patricia.hansen@fda.hhs.gov>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>
Subject: FW: list

Ikhlas,

Attached is the list of the 26 EU fragrance allergens as well as the SCCS opinion. Some of these substances are either confirmed contact allergens or presumptive contact allergens (but not confirmed); other tables present those which are suspected to be human contact allergens or which are based on animal, in-vitro (LLNA), or QSAR/*in-silico* data. Table 13-5 presents 12 established chemical fragrance allergens with high risk of sensitization to humans. In the SCCS Opinion, the Threshold exposure (for single chemicals only, not extracts or mixtures) which could be tolerated by most consumers is estimated to be = 0.8 µg/cm².

Chemical of special concern include: tree moss, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), oak moss, isoeugenol, hydroxycitronellal, citral, cinnamal, farnesol and cinnamyl alcohol. However, we are interested in the remainder of those listed below from Table 13-5.

Cinnamal
Cinnamyl Alcohol*
Citral
Coumarin
Eugenol*
Farnesol*
Geraniol*
Hydroxycitronellal
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)
Isoeugenol*
Limonene (oxidised)
Linalool* (oxidised)
*including their respective esters

The 2012 SCCS Opinion also recommends that fragrance pre-haptens and pro-haptens of several terpene fragrance materials should be considered as putative “allergens” and regulated in the same way as the allergens by the EC (Among them are limonene, linalool, linalyl acetate, geraniol, a-terpinene, eugenol, isoeugenol, and cinnamyl alcohol).

Let me know if you need any further information at this time regarding allergens.

On a different note, let me know if you have a report and list with description of in vitro assays, including validation, that actually describes the methods that you have developed for arbutin.

Linda

From: Hansen, Patricia A
Sent: Wednesday, June 17, 2015 1:21 PM
To: Ikhlas Khan
Cc: Katz, Linda
Subject: RE: list

Hi, Ikhlas. Sorry not to get back to you sooner.

I think the conversations we had while on our visit were very helpful.

By copy of this note, I'll alert Linda to your request for the fragrance allergen list. We talked about it during our visit and she may have assigned it to someone already, but I'm not sure.

Is there a conference call already scheduled, perhaps with the dietary supplement group and others or are you talking about something else?

Hope all is well.

PH

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Friday, June 05, 2015 10:58 AM
To: Hansen, Patricia A
Subject: list

Hi Pat

I know this is your first week and must be busy. Could you please share the list of 26 and may be 80 if possible.

It wil help to us to see what is coming but you will still have time to prioritize before we have conference call

Thanks

IK

Application of UM's *in chemico* methods for Estimating the Sensitization Potential

Case studies on Chamomile and Tea Tree Oil



Case Study#1

Skin sensitization potential of chamomile



NOPSIS

Two most popular and commonly used chamomiles

German chamomile (*Matricaria chamomilla*, synonym: *Matricaria recutita*)

Roman chamomile (*Chamaemelum nobile*, synonym: *Anthemis nobilis*)



Matricaria chamomilla



Chamaemelum nobile

➤ **Aim:**

1. Which chamomile? Roman, German or Chinese?
2. Skin sensitization potential?

➤ **Methodologies applied:**

GC-MS

UPLC-QToF

HPTLC

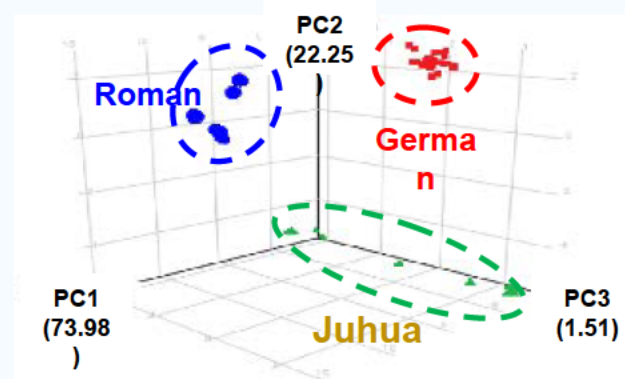
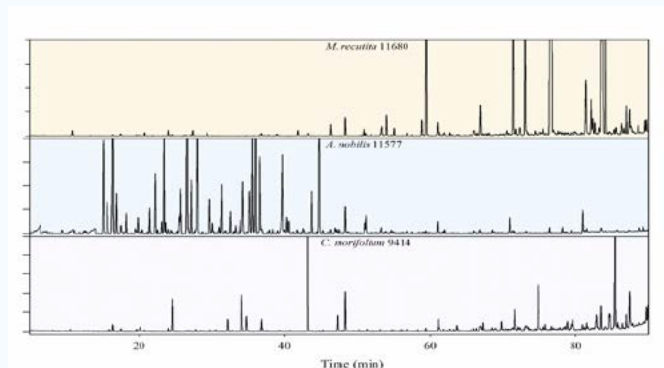
HTS-DCYA

NMR-DCYA

KeratinoSens™

Classification of Chamomiles Using GC/MS and Chemometrics

➤ Results:



- A highly accurate statistical model has been developed to determine the exact type (German, Roman and Juhua) of chamomile used in commercial herbal products and dietary supplements.
- The model was developed from GC/MS data. Quality control of the samples was performed by Principal Component Analysis (PCA).
- A sample class prediction model based on Partial Least Squares Discriminant Analysis (PLS-DA) was constructed.
- The results suggested that German chamomile is the major type of chamomile used in the U.S. market.

Classification of Chamomiles Using UHPLC-UV-MS

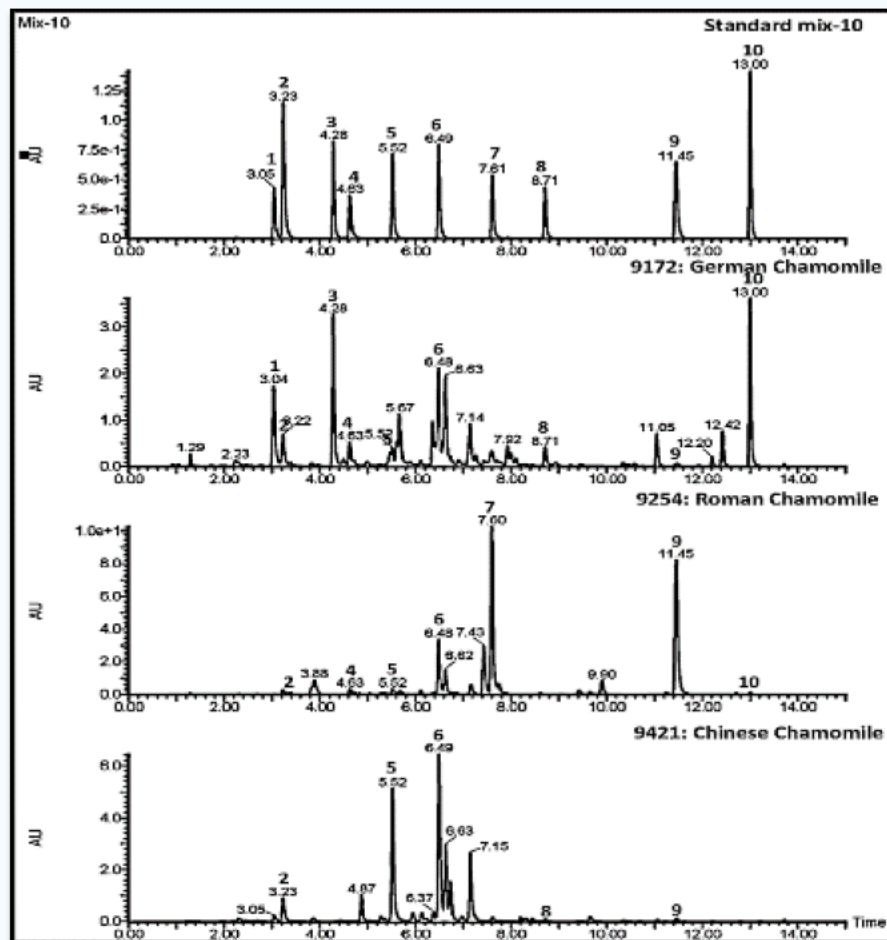
Results:

Standard Mix-10

German chamomile

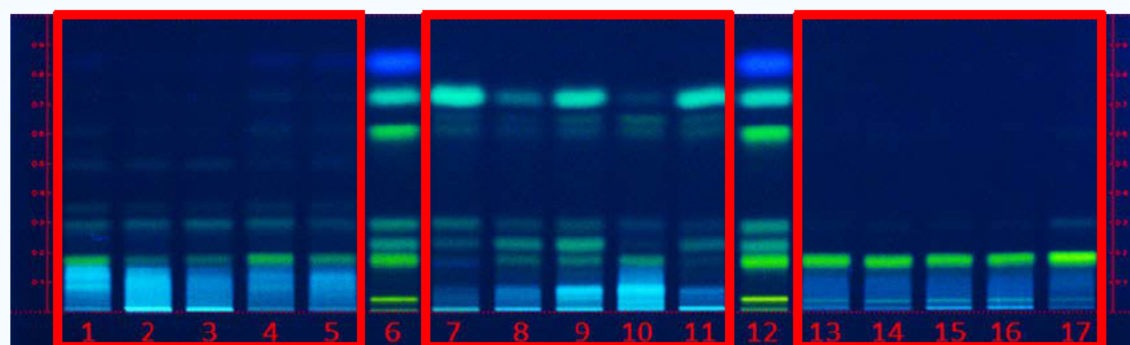
Roman chamomile

Chrysanthemum morifolium



Classification of Chamomiles Using HPTLC

➤ Results:

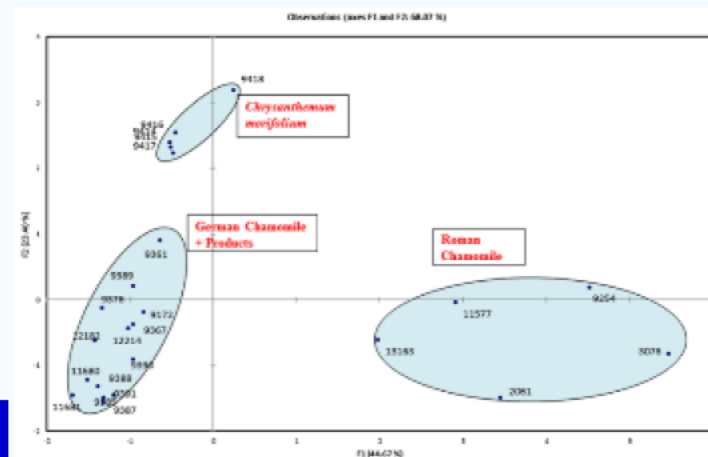


German
chamomile

Roman
chamomile

Juhua

Reference standards (Rf order): rutin (1), luteolin-7-O-glucoside (2), chamaemeloside (3), apigenin-7-O-glucoside (4), luteolin (5), apigenin (6), and umbelliferone (7)



PCA score plots for authenticated chamomile samples and *Chrysanthemum*

Determination of Sensitization Agents Within Chamomile Species

➤ Results: KeratinoSens™ data

EC1.5 and IC₅₀ Summary Table

Assay Date	HIVS Test Article	Sponsor Designation	EC 1.5 value [^] (μg/mL)	Mean IC ₅₀ (μg/mL)		Potential Sensitizer? [†]
				MTT	NRU	
	11AH38	German Chamomile Hexane Fraction (9172 Hexane, JZ-11A-2-2)	0.67	168	160	YES
	11AH39	German Chamomile Chloroform Fraction (9172 CHC13, JZ-11A-13-2)	5.85	155	72.6	YES
	11AH40	German Chamomile Ethanol extract (3760 EtOH, IKX-1-55.11)	21.23	> 400	> 400	YES
26 July 2011	11AH41	Roman Chamomile Hexane Fraction (9254 Hexane, JZ-11A-10-2)	2.66	88.5	81.4	YES
	11AH42	Roman Chamomile Chloroform Fraction (9254 Hexane, JZ-11A-10-3)	0.50	9.96	8.10	YES
	11AH43	Chamomile Essential oil (9369)	2.23	9.47	9.44	YES
	11AH44	Bisabolol	> 400	9.38	9.30	NO
7 Sept 2011	Chloroform	Chloroform	> 400	> 400	> 400	NO
	Hexane	Hexane	> 400	> 400	> 400	NO
26 July 2011	Cinnamic Aldehyde	Positive Control	10.26 μM	> 64 μM	> 64 μM	YES
7 Sept 2011			8.16 μM	> 64 μM	> 64 μM	YES

EC1.5 and IC₅₀ Summary Table

Assay Date	HIVS Test Article Number	Sponsor's Designation	EC 1.5 value [^] (μg/mL)	Mean IC ₅₀ (μg/mL)		Potential Sensitizer? [†]
				MTT	NRU	
	12AE99	JZ-12-11-3, German Chamomile Extract	3.29	>400	377	YES
	12AF00	JZ-12-15-1, German Chamomile Fraction	2.96	291	352	YES
	12AF01	JZ-12-14-3, German Chamomile Fraction	3.02	336	>400	YES
15 May 2012	12AF02	JZ-12-14-4, German Chamomile Fraction	0.471	>400	>400	YES
	12AF03	JZ-12-14-5, German Chamomile Fraction	24.8	279	290	YES
	12AF04	JZ-12-14-6, German Chamomile Fraction	34.8	>400	>400	YES
	12AF05	JZ-12-7-3, Roman Chamomile Extract	0.718	83.5	80.7	YES
	Cinnamic Aldehyde	Positive Control	13.3	>64	>64	YES

Determination of Sensitization Agents Within Chamomile Species

➤ Results:

- German and Roman Chamomile extracts along with fractions were evaluated for sensitization potential using KeratinoSens™ assay by a commercial laboratory (IIVS).
- Several fractions are found to have sensitization potential without any cytotoxicity.
- Direct Peptide Reactivity Assay failed to estimate the sensitization potential of the pure compounds of German Chamomile due to their solubility in only DMSO. The solvent, DMSO was found to have detrimental effects on Cys-DPRA results.

Determination of Sensitization Agents Within Chamomile Species

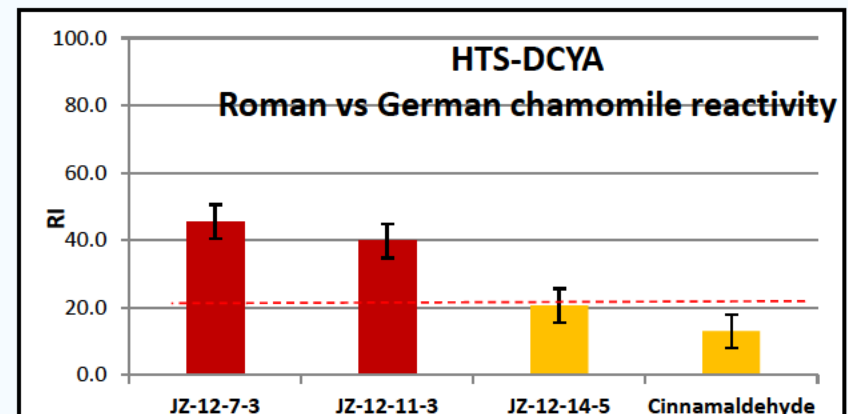
➤ Results:

- Authenticated German (*Matricaria recutita*) and Roman chamomile (*Chamaemelum nobile*) samples were screened with DCYA-HTS method and compared with KeratinoSens™ data.
- Roman chamomile (RC) crude extract resulted in a stronger response compared to German chamomile (GC) extract and enriched fractions
- The results obtained with the DCYA-HTS method were comparable to in vitro results

Sample	Description	HTS	Keratinosens ¹
		RI*	IC ₅₀
JZ-12-7-3	Roman Chamomile Extract	45.5	0.718
JZ-12-11-3		39.8	3.29
JZ-12-14-5	German Chamomile Fraction	20.6	24.8
Cinnamaldehyde	Positive control	14.9	13.3

*Note: for HTS results, the higher the RI, the stronger the sensitizer

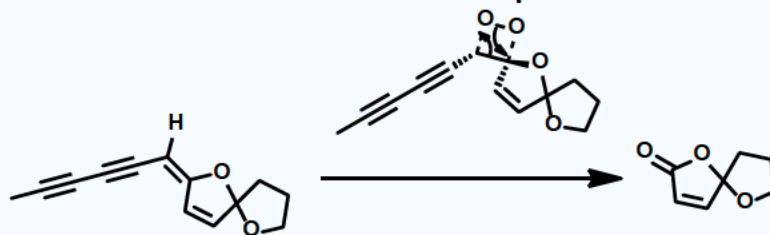
¹See Report Jul 31st 2013- study number 12AE99-AF05.170000, Institute for In Vitro Sciences,



Determination of Sensitization Agents Within Chamomile Species

➤ Results:

- **Tonghaosu** is one of the main components of **German** chamomile
- The compound possesses several structural alerts and can be considered as a potential hazard as it has **structure resemblance to known sensitizers** (e.g. falcarinol)¹
- Tonghaosu was **non-reactive** under DCYA-NMR and DCYA-HTS methods
- Identified unstable nature of tonghaosu, isolated oxidative degradation product and characterized as dioxo-spiro lactone.

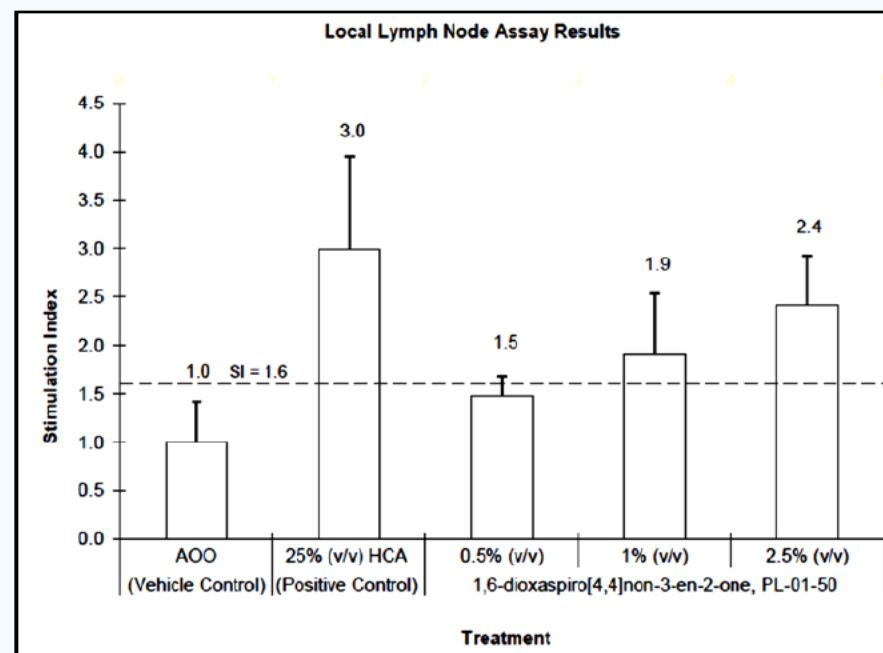


Determination of Sensitization Agents Within Chamomile Species

➤ Results:

LLNA data

- In order to confirm the sensitization potential of the identified lactone, several grams of the compound was synthesized to meet the sample requirements for LLNA. In vivo studies were performed by an external laboratory¹
- The compound was tested at different concentrations, and classified as a dermal sensitizer



¹MB Research Laboratories, Research Project MB # 14-23242.26

Determination of Sensitization Agents Within Chamomile Species

➤ Outcomes :

- According to WHO monogram on German chamomile, very few cases of allergy were specifically attributed to German chamomile.¹ Adverse effects were attributed to presence of Lactones
- Tonghaosu is one of the major marker compound in essential oil of German chamomile
- Several commercial EOs of German chamomiles were studied and 50-160 mg of Tonghaosu per gram of EO was observed.
- Tonghaosu undergoes oxidative transformation into potential sensitizer (dioxo-spiro lactone) and can be considered a pre-hapten. The sensitization potential was confirmed with LLNA.

¹Hausen BM, Busker E, Carle R. Über das Sensibilisierungsvermögen von Compositenarten. VII. Experimentelle Untersuchungen mit Auszügen und Inhaltsstoffen von Chamomilla recutita (L.) Rauschert und Anthemis cotula L. *Planta medica*, 1984:229–234.

Case Study #2

Authentication and safety concern of Tea Tree Oil



SYNOPSIS

- Essential oil obtained by steam distillation of the foliage and terminal branch lets of *Melaleuca alternifolia*, *Melaleuca linariifolia*, *Melaleuca dissitiflora* or other species of the genus *Melaleuca*
- Due to the increasing market for Tea Tree Oil (TTO), there is a growing trend toward adulteration or substitution of these products
- A total of 104 samples were provided by ATTIA Ltd (Australia) and analyzed for authenticity
- *In vivo* studies (LLNA) confirmed a stronger sensitization potential for aged TTOs compared to fresh ones. A number of constituents of TTO have been suggested as potential candidate sensitizers due to chemical or metabolic transformation in *in vivo*
- Ten different TTO and related species have been investigated for the sensitization potential after aging along with major components of TTO

➤ **Aim:**

1. Development of analytical methods to distinguish authentic TTO from other tea tree species
2. Investigation of the sensitization potential of TTO and related species using HTS-DCYA assay with crude EO mixtures
3. Investigation of the sensitization potential upon aging of individual TTO main constituents

➤ **Methodologies applied:**

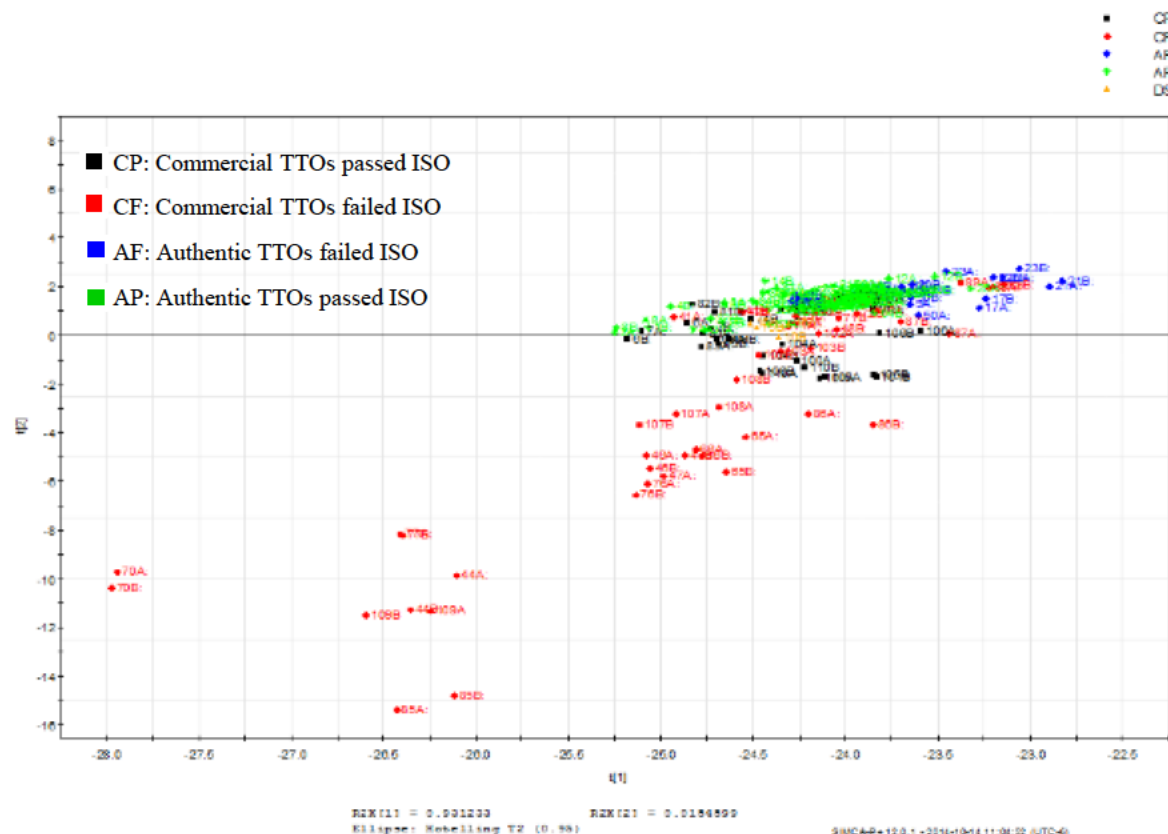
- GC-MS coupled to PCA
- HTS-DCYA

Identification of the Major Compounds in Tea Tree Oil

Peak No.	Compound	t _R	%
1	α -thujene	16.10	1.01
2	(-)- α -pinene	18.67	0.27
3	(+)- α -pinene	19.05	3.02
4	β -myrcene	19.85	0.44
5	β -pinene	21.97	0.52
6	α -terpinene	22.12	5.83
7	p-cymene, (\pm) limonene	23.26	25.07
8	(\pm)- β -phellandrene	24.01	0.21
9	(\pm)- β -phellandrene	24.19	0.37
10	γ -terpinene	24.78	17.27
11	α -terpinolene	25.73	5.61
12	1,8-cineole	26.07	2.88
13	(+)-terpinen-4-ol	34.64	16.35
14	(-)-terpinen-4-ol	34.53	9.36
15	(-)- α -terpineol	36.41	0.36
16	(+)- α -terpineol	36.56	1.25
17	α -copaene	37.07	0.20
18	p-cymene-8-ol	37.93	0.29
19	α -gurjunene	39.03	0.20
20	β -caryophyllene	40.65	0.25
21	alloaromadendrene	41.66	0.85
22	aromadendrene	42.61	0.32
23	viridiflorene	44.50	0.69
24	δ -cadinene	46.02	1.19
25	globulol	51.92	0.51
26	viridiflorol	52.45	0.58
Total			94.87

PCA analysis for authentication of TTO

- All the authentic TTOs that met the ISO standards cluster in a single group (green) in the PCA score plot.
- The chiral ratios of α -pinene, limonene, terpinen-4-ol and α -terpineol for the tea tree oil samples in this group were also within the ranges of norms.



Identification of the Major Compounds in Tea Tree Oil

➤ Outcome :

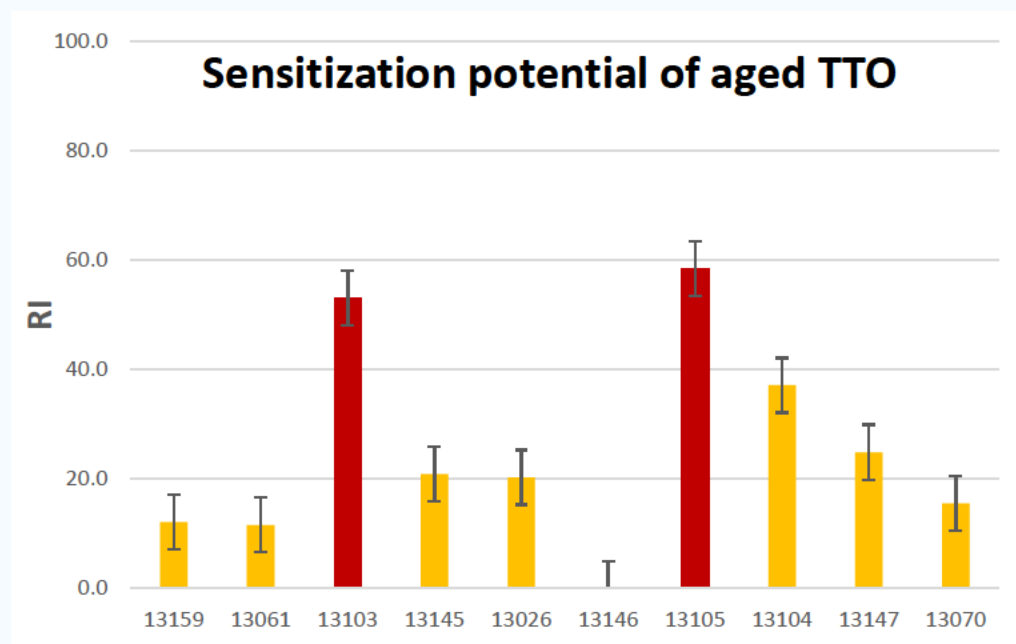
- A GC/MS method was developed for the analysis of the enantiomeric compounds in tea tree oil.
- A total of 104 samples from ATTIA Ltd., Australia were acquired. The ratios of four enantiomeric compounds were determined.
- The distribution of enantiomeric ratios were utilized for establishment of quality and authenticity of the tea tree oil.
- Based on GC-MS data, Principle Component Analysis was conducted and a model was developed for establishing the authenticity of TTO. The use of a chemometric tool, such as PCA, can produce complementary information to chiral GC.

Sensitization Potential of TTO

- Ten TTO samples of *M. alternifolia* and related species previously analyzed by our group were undertaken for the aging study
- Aged samples were then characterized by GC/MS and compared data to the original chemical composition
- The content of *p*-cymene and hydrogen peroxide were quantified as indication of aging
- The aged oils were then assessed for their sensitization potential using the DCYA-HTS method.

Sensitization Potential of TTO

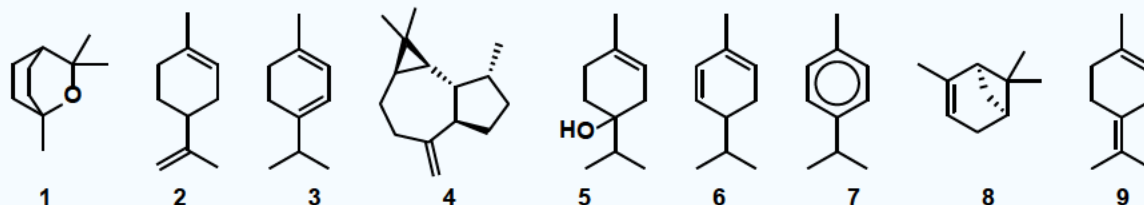
- From the DCYA-HTS results, some oils resulted stronger reactive indices indicating the higher sensitization potential
- With regard to authentic TTO (*M. alternifolia* and *M. linariifolia*), a correlation between content of *p*-cymene and sensitization potential was found



	PLANT SOURCE
# 13159	Melaleuca alternifolia
# 13061	Melaleuca cajuputi
# 13103	Melaleuca leucadendron
# 13145	Melaleuca
# 13026	Melaleuca linariifolia
# 13146	Leptospermum scoparium
# 13105	Melaleuca alternifolia
# 13104	Melaleuca viridiflora
# 13147	Kunzea ericoides
# 13070	Leptospermum + Melaleuca

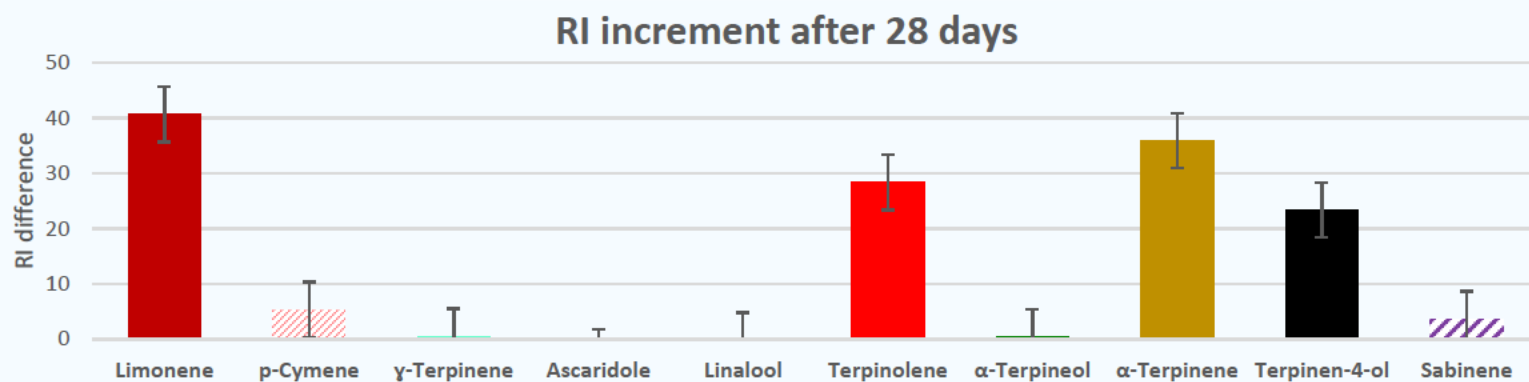
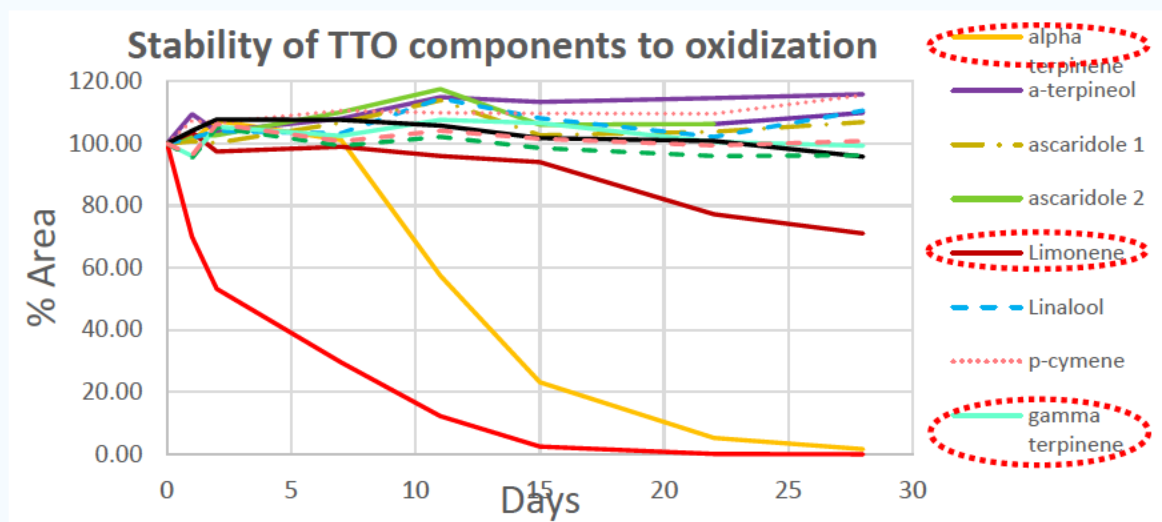
Sensitization Potential of TTO's Components

- As a clear correlation between the monoterpenes content, aging and potential sensitization was found, the principal TTO components (1-9) were also investigated for their sensitization potential



- The pure compounds were subjected to expedited aging by continuous bubbling of oxygen for 28 days.
- Several aliquots were collected (0-28 days), analyzed for their stability with GC/MS and subjected to UM-HTS method for their sensitization potential
- Terpinolene**, **α -terpinene** and **limonene** were found to be most unstable under these conditions

Sensitization Potential of TTO's Components



Sensitization Potential of TTO and its Major Components

➤ Outcomes:

- GC-MS method and PCA model were developed for to establish the authenticity of Tea Tree Oils
- A strong correlation between aging and sensitization potential of genuine TTOs was observed
- Non-aged pure TTO components were found non- to weakly reactive
- Upon accelerated aged conditions, increased sensitization potential was observed and the chemical reactivity is proportional to the degradation ability of each component.
- The obtained results are in agreement with the literature reports on skin sensitization potential of oxidized TTO and its components.
- The DCYA-HTS method served as a useful tool in estimating the sensitization potential of complex essential oils, viz., Tea Tree Oils

TOOLS FOR ESTIMATION OF SKIN SENSITIZATION POTENTIAL

METHODS AVAILABLE AT UM

EX VIVO ASSAYS AVAILABLE AT UM

IN CHEMICO

Spectrophotometric High Throughput Screening method (HTS-DCYA)

Nuclear Magnetic Resonance (NMR) spectroscopic method (NMR-DCYA)

IN VITRO

Human Cell Line Activation Test (h-CLAT)

HTS-DCYA ASSAY

Fluorescent spectrophotometric method recently developed at UM¹

Features

The developed method is ideal for the *simultaneous* analysis of multiple test articles

Mixtures of multiple components can also be *qualitatively* analyzed for the content of reactive components

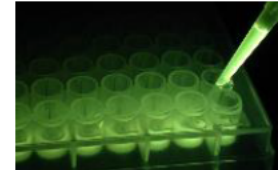
Advantages

- ✧ Minimum sample requirements
- ✧ Comparable to methods approved by regulatory agencies (Cys-DPRA)
- ✧ Ideal for pre-screening of mixtures for Substances of Concern (SoC)

Limitations

- Pre- or pro- sensitizers may require chemical/metabolic activation before the testing

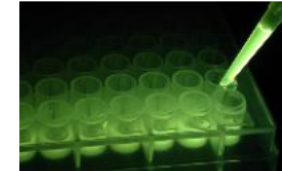
¹U.S. Provisional Application Serial No. 62/017,586; Manuscript under review, TAAP



HTS-DCYA ASSAY

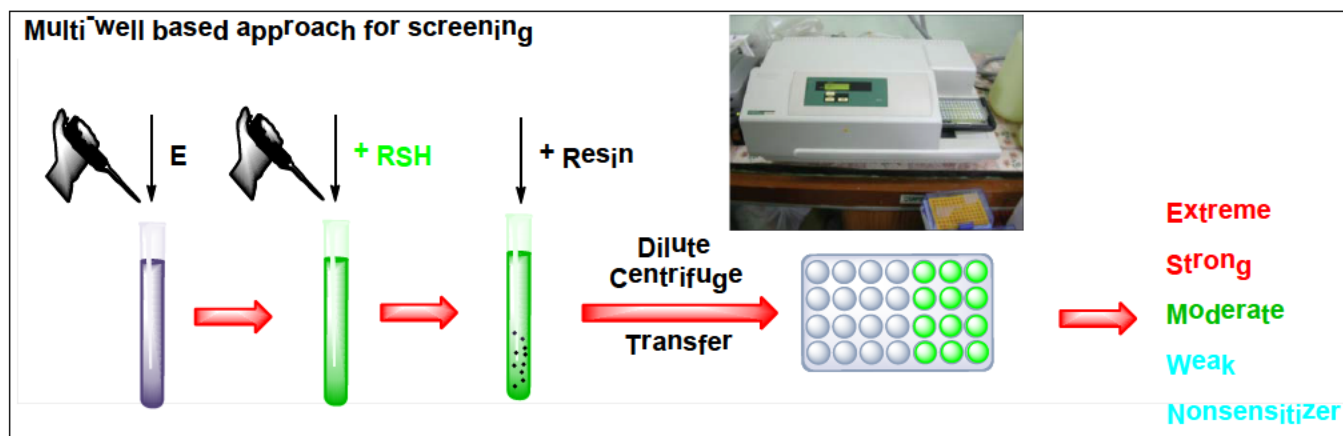
Description

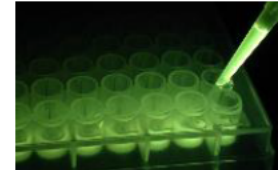
- *In chemico* method based on the reaction of the candidate sensitizer (test article) with a model fluorescent nucleophile (DCYA)
- After incubation, the unreacted nucleophile is removed and the fluorescent adduct, DCYA-sensitizer, is quantified
- The fluorescence response is directly proportional to the reactivity of the potential sensitizer; i.e., strongly reactive sensitizers result high fluorescence response, whereas non-sensitizers would result no-to-minimal fluorescence response.



HTS-DCYA ASSAY

Flow diagram of spectrophotometric method





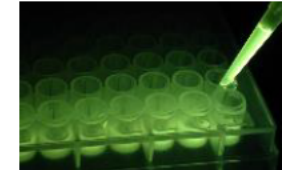
HTS-DCYA ASSAY

Method validation

- A total of 33 compounds* were chosen for the validation of method (HTS-DCYA) described in the previous slides
- Pre-/pro-haptens, non-sensitizers, weak, moderate and strong/extreme sensitizers have been included
- Based on Reaction Indices, further binary analysis was applied for comparison with approved *in chemico* methods (*viz.*, Cys-DPRA)**

*The list of compounds used for the method validation included Diphenyl cyclopropanone, *p*-Benzoquinone, 1-Chloro-2,4-dinitrobenzene, *p*-Hydroquinone, Propionolactone, 3-Hydroxytyrosol, 1,2-cyclohexanedicarboxylic anhydride, 2-Methyl-4-isothiazolin-3-one, Cinnamaldehyde, 2,4-Heptadienal, 4-Hex-3-en-one, Squaric acid, *t*-2-Hexenal, Resorcinol, Diethyl maleate, Safranal, Perillaldehyde, Citral, Farnesal, L-Carvone, Oxalic acid, Benzyl Benzoate, Lilial, Cinnamyl alcohol, *cis*-6-Nonenal, 5-Methyl-2,3-hexanedione, Ethyl acrylate, Aniline, 1-Bromobutane, Vanillin, Tartaric acid, Chlorobenzene, Lactic acid, Salicylic acid, Coumarin, Benzaldehyde

**Statistical analysis excluded pre-/pro-haptens



HTS-DCYA ASSAY

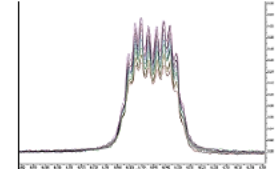
Cooper statistic analysis

	DCYA	Cys-DPRA*
True Positive	18	17
True Negative	9	8
False Positive	1	2
False Negative	5	0
Total articles	33	27

*Cys-DPRA results were taken from the literature

	DCYA	DPRA
Accuracy	81.8	92.6
Sensitivity	78.3	100.0
Specificity	90.0	80.0

- ✓ Based on the limited sample set, the HTS-DCYA results were comparable with the recently approved Cys-DPRA
- ✓ Less false positives due to limited auto-oxidization of the nucleophile



NMR-DCYA ASSAY

Spectroscopic method recently developed at UM based on nuclear magnetic resonance¹

Features

Ideal for the *direct quantification of reaction adducts* by NMR spectroscopy

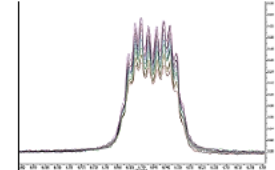
Advantages

- ✧ Multifaceted method allows monitoring of the depletion of test articles, nucleophile and/or formation of adducts
- ✧ Direct quantification of reactivity and can be applied to improve *in silico* predictions
- ✧ Ideal for *structural, molecular and mechanistic analysis*, to identify the reaction site in the presence of multiple mechanistic domains

Limitations

- One sample at a time, time-consuming and less sensitive than HTS-DCYA
- Pre- or pro- sensitizers may require chemical/metabolic activation before screening

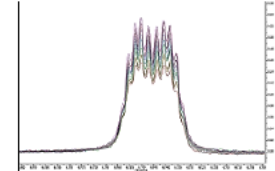
¹U.S. Provisional Application Serial No. 62/017,586; Manuscript under review, *Chem Res Toxicol*



NMR-DCYA ASSAY

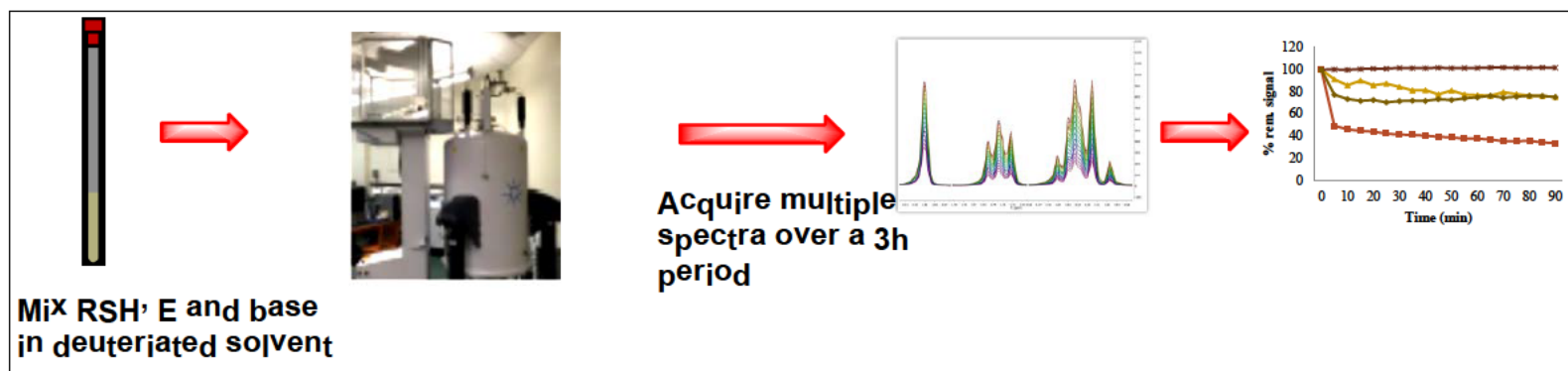
Description

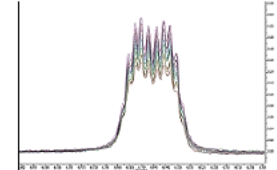
- *In chemico* spectroscopic method based on the quantification of test article/nucleophile depletion and/or formation of adduct (test article-DCYA) by nuclear magnetic resonance
- The candidate sensitizer (test article) is mixed with the model nucleophile (*e.g.* DCYA). The reaction is monitored by acquiring ^1H -NMR spectrum at regular intervals for 3 h
- The reaction is quantified by calculating the variation of distinctive resonance signal(s) of the sensitizer and/or nucleophile and/or by monitoring signals corresponding to the adduct
- The rate of depletion is directly proportional to potency of the given test article



NMR-DCYA ASSAY

Flow diagram of NMR method



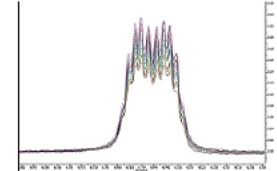


NMR-DCYA ASSAY

Method validation

- A total of 17 compounds* were chosen for the validation of method described in the previous slides
- Pre-/pro-haptens, non-sensitizers, weak, moderate and strong/extreme sensitizers have been included

*The list of compounds used for the method validation included *p*-Benzoquinone, Ethyl acrylate, *p*-Hydroquinone, 3-Hydroxytyrosol, Cinnamaldehyde, Safranal, Perillaldehyde, Citral, L-Carvone, Coumarin, Nootkatone, Curcumin, Massoia lactone, 2-pentenal, Parthenolide, Costunolide, Alantolactone



NMR-DCYA ASSAY

Method validation*

	LLNA/PATCH TEST	NMR-DCYA
Non-sensitizer	1	2
Weak	0	2
Moderate	5	2
Strong/extreme	5	5
Tot. positive	10/11	9/11
Tot. negative	1/11	2/11

*Pre-/pro-hapten were excluded from the statistical analysis

IN VITRO

hCLAT

Features

- *In vitro* method based on expression of CD86 and CD54
- THP-1 cell lines are used as a substitute for human dendritic cells (DC)

Advantages

- ✧ *In vitro* assay
- ✧ Recently validated by European Regulatory agencies

Limitations

- Costly
- Time-consuming
- Cell based assay, testing cytotoxic component(s) is problematic
- Low throughput (3 samples/assay)

IN VITRO

hCLAT

Description

- Dendritic cells (DC) activation is one of the major steps of the induction phase of skin sensitization
- During the process, DC change from antigen processing to antigen presenting cells and exhibit up regulation of expression CD86 and CD54
- The hCLAT method is based on the expression of CD86 and CD54 protein markers on the surface of the human monocytic leukemia cell line (THP-1), following exposure to test chemicals

IN VITRO

hCLAT

Experimental design



Pre-culture THP-1 cells for 48-72 hours

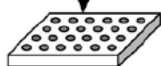
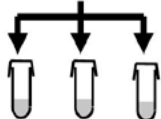


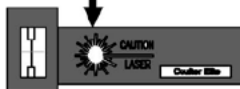
Plate (1×10^6 cells/well) in 24-well plate, treat with test chemical for 24 hours



Harvest cells, wash and block FcR (0.01% Globulins) for 15 min.



Divide cells into 3 aliquots, stain with FITC-conjugated monoclonal antibodies (isotype control, CD86, CD54) for 30 min.



Analyze by flow cytometry - mean fluorescence intensity of CD86 and CD54, cell viability by propidium iodide exclusion.

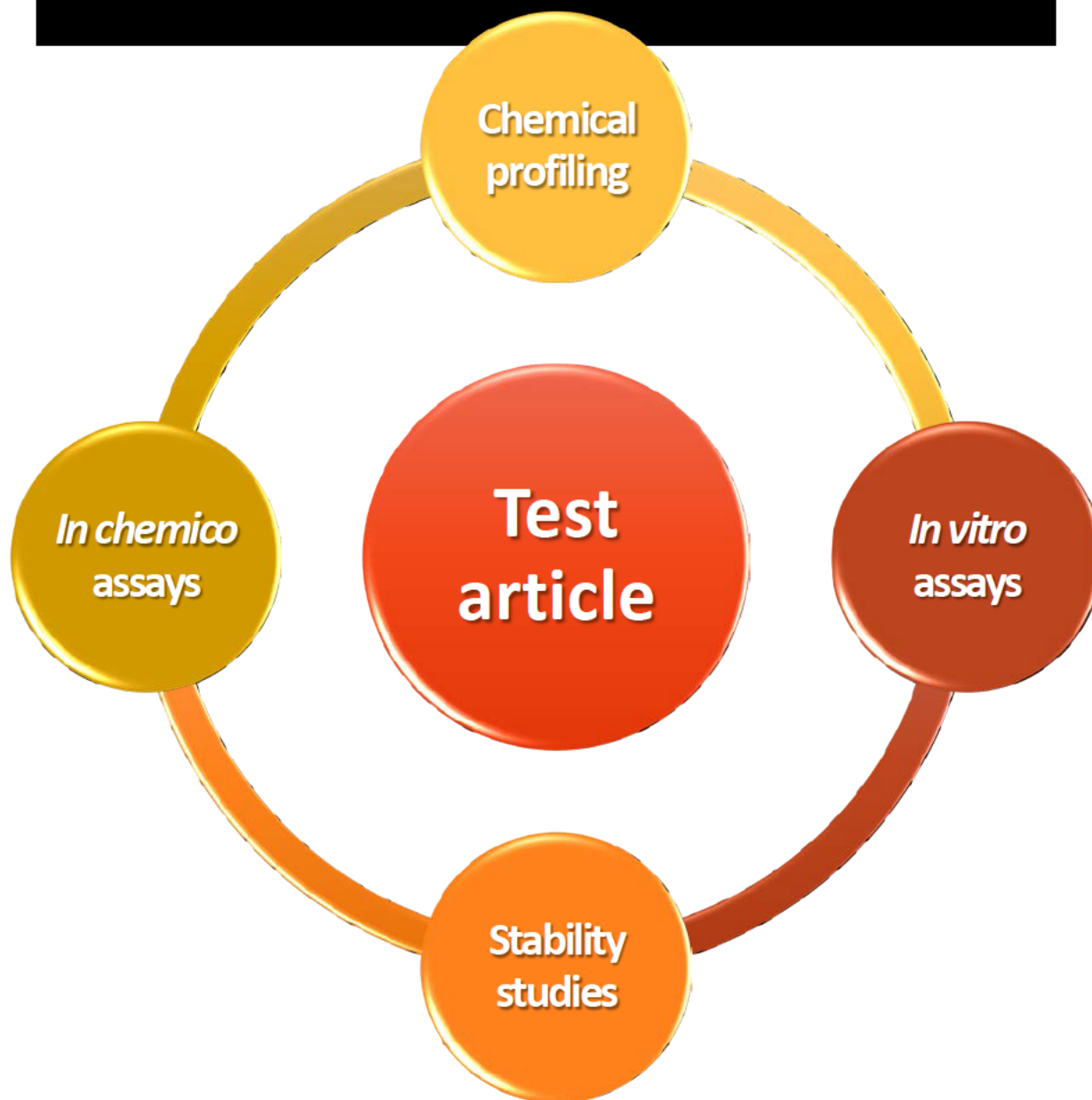
Two of three independent measurements at any dose should exceed the positive criteria (CD86 >150% or CD54 >200%) in order to be judged as positive.

Status

- The method has been optimized
- Validation with know sensitizers is in progress

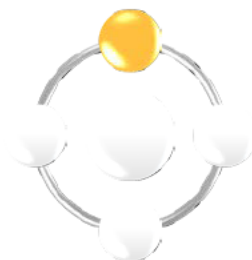
RISK ASSESSMENT OF SENSITIZERS IN SKIN CARE PRODUCTS

26 FRAGRANCE ALLERGENS



26 FRAGRANCE ALLERGENS

CHEMICAL PROFILING

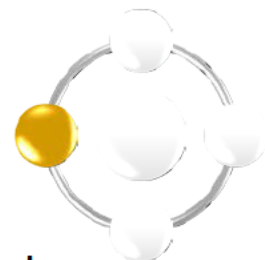


- Is there any known Substance of Concern (SoC)?
 - ✓ Analytical investigation for 26 fragrance allergens
 - ✓ Assessment of the presence of other suspects allergens (see extended list of 80 chemicals)
- Is there any potential *source* of SoC?
 - ✓ Presence of congeneric groups?
 - ✓ Chemically related compounds?
 - ✓ Stability issues?

26 FRAGRANCE ALLERGENS

IN CHEMICO ASSAYS

- Is there any known/unknown potential SoC?
 - ✓ Assessment of the sensitization potential using chemical reactivity based assays
- How many SoC are present?
 - ✓ Assessment of mixtures for the overall sensitization potential in relationship to the major components
 - ✓ Formulation effects?



26 FRAGRANCE ALLERGENS

STABILITY STUDIES

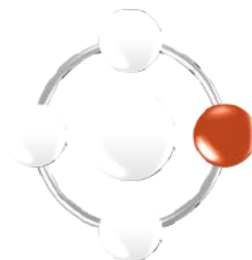
- Is there any chemical activation?
 - ✓ Air oxidization?
 - ✓ Light stability?
 - ✓ Reactive species formation?
 - ✓ pH stability?
- How many SoC are present?
 - ✓ Assessment of mixtures for the overall sensitization potential in relationship to the major components
 - ✓ Formulation effects?



26 FRAGRANCE ALLERGENS

IN VITRO STUDIES

- Identified selected articles and SoC can be further investigated using *in vitro* assays available at UM
 - ✓ hCLAT
- Integrated Strategy (both *in chemico* and *in vitro* data for same test article) for estimating the potency of potential allergens
 - ✓ Data integration and harmonization?
 - ✓ Better predictability and reliability?
- Inter-laboratory validation for both *in chemico* methods



BOTANICALS FOR SKIN CARE

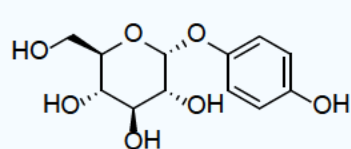
Current status

- ✓ *In chemico* methods developed at UM are currently under optimization for the screening of mixtures, plant extracts and botanicals of cosmetic relevance and pre-haptens
- ✓ A list of fragrances and natural constituents currently under evaluation includes limonene, linalool, α -terpinene, γ -terpinene, terpinolene, *p*-cymene, ascaridole, jasmone, citral, coumarin, cinnamaldehyde
- ✓ A list of plant extracts currently under evaluation with UM_HTS method includes lavender, German and Roman chamomile, *Arnica montana*, *Taraxacum officinale*, *Cinnamomum* spp., *Citrus* spp., *Cymbopogon* spp., *Calendula officinalis*, *Tanacetum parthenium*, *Hamamelis virginiana*, *Pogostemon cablin*

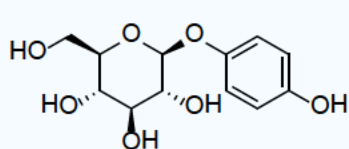
Synopsis on OCAC's projects

ARBUTIN

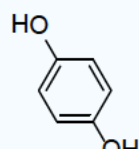
- ❖ Arbutin is the glycoside derivative of hydroquinone. The β -glycoside anomer is commonly found in species of several plant families in nature, whereas commercial α -arbutin is usually obtained by biotransformation or by chemical synthesis
- ❖ Both β - and α -arbutin became popular skin whitening agents because of their ability to interfere with the melanin synthesis
- ❖ Because of their structural similarity to hydroquinone, both arbutins can be regarded as potential sources of the compound of concern hydroquinone upon topical application
- ❖ Other popular “natural” whitening agents include kojic acid, nicotinamide, ascorbic acid and resorcinol



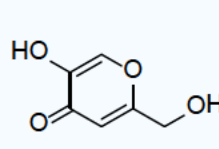
β -Arbutin



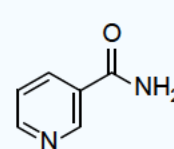
α -Arbutin



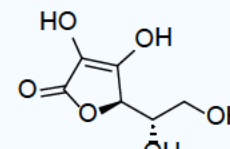
1,4-Hydroquinone



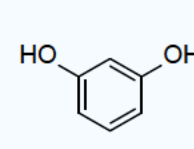
kojic acid



nicotinamide



ascorbic acid



resorcinol

TASKS

✓ **Task #1:**

Natural occurrence of arbutins in plant species

✓ **Task #2:**

Inter-conversion and stability (chemical and *ex vivo*)

✓ **Task #3:**

Distribution in cosmetics including the development of an LC method for detecting arbutin and commonly found whitening ingredients

Natural occurrence of arbutins in plant species

➤ **Aim:**

Investigation of distribution of β - and α -arbutin in plants

➤ **Method developed:**

- 1) Extraction procedure for the analysis of arbutins in plants
- 2) HPLC-UV method

➤ **Experiments performed:**

HPLC screening

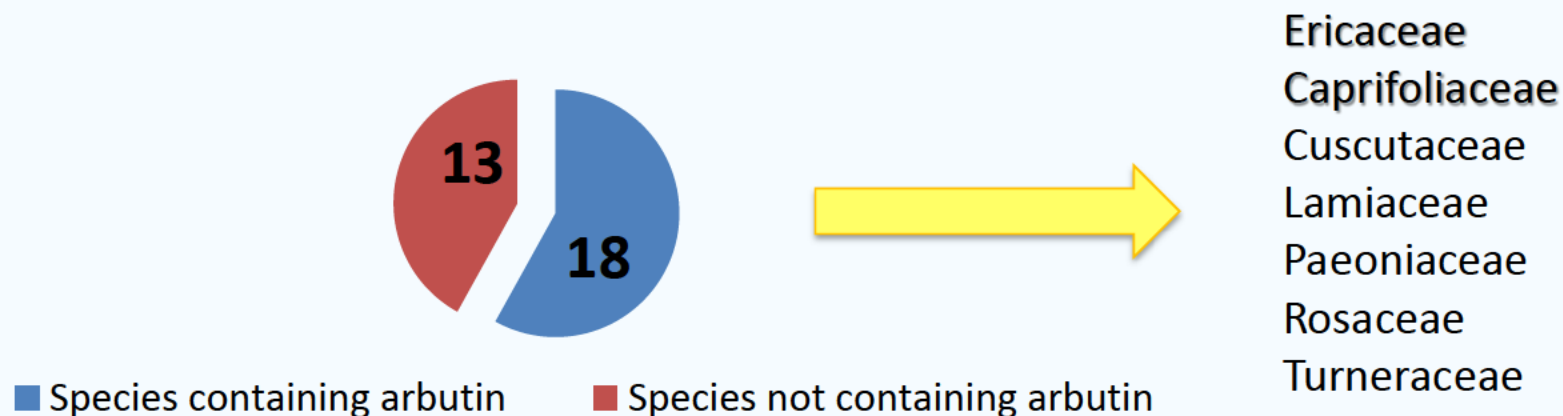
TASK #1



THE UNIVERSITY OF
MISSISSIPPI
National Center for
Natural Products Research

➤ Results:

A total of 59 specimens covering 31 species, (22 genera and 19 families) were analyzed for the occurrence of both arbutins in different part plants



➤ Conclusions:

Eighteen species were found to contain β -arbutin, while none was found as positive source of α -arbutin

Chemical and enzymatic stability of arbutins

➤ **Aim:**

Assessing the stability of arbutins with regard to the potential release of hydroquinone or epimerization in conditions of potential relevance for cosmetics

➤ **Experiments performed:**

HPLC screening

Nuclear Magnetic Resonance (NMR)

Optical Rotation (OR)

Experimental procedures

- Sixteen months stability study on 9 commercial arbutins preparations (sera, creams, lotions and gels) with different formulation and pH characteristics (method used: HPLC-UV)
- NMR/OR studies for stability of arbutins in solution in absence of additives or buffers
- Metabolic stability studies using pear peels as surrogate for skin metabolism and HPLC-UV method

Results

- The stability of both arbutins was lower in liquid preparations. The less stable formulation was a serum (pH 8.8), resulting in 20% loss of β -arbutin after 16 months
- Creams and semi-solid formulations were relatively stable, with major loss of 8% of β -arbutin in one formulation having pH 3.3
- Both arbutins were found to be stable in aqueous/methanol solutions but unstable to strong hydrolytic conditions (2.5 h at 100 °C, pH 1.1)
- Both arbutins were rapidly metabolized in less than 8 h using pear peels as a surrogate *ex vivo* model.

Conclusions

- Both compounds presented similar stability profiles under selected chemical or biological conditions
- The stability in cosmetic formulations may be strongly dependent on the type of formulation and the pH
- Liquid formulations may be less stable than semi-solid preparations
- Both arbutins were stable in water or methanol up to 12 months with no generation of hydroquinone
- Both arbutins rapidly generate hydroquinone under strong hydrolytic conditions
- Both arbutin were unstable when exposed to enzymatic metabolism using pear peels as a source of β -glucosidases and peroxidases (known to regulate the accumulation of HQ/ARB *in vivo*)
- Hydroquinone was not found as a degradation product of arbutins after metabolic degradation
- Anomerization products were not found under any of the studied experimental conditions

Investigation of whitening agents in cosmetics

- **Aim:**

Investigation of the distribution of common whitening agents in cosmetic formulations
- **Method developed:**

Sample preparation for the simultaneous different types of skin whitening agents
HPLC-UV method for the simultaneous detection of 9 analytes
- **Experiments performed:**

HPLC-UV screening
- **Results:**

Fifty-nine skin whitening products including creams, lotions, sera, foams, gels, mask sheets, soap bars, tablets, and capsules were analyzed for the presence of common natural whitening agents, including α -arbutin, β -arbutin, kojic acid, nicotinamide, resorcinol, ascorbic acid, hydroquinone, and hydroquinone derivatives (4-methoxyphenol, and 4-ethoxyphenol)

Conclusions:

- The newly developed method enabled a baseline separation of nine analytes within 30 min.
- Arbutins (β - and/or α -), ascorbic acid, kojic acid, nicotinamide, hydroquinone, and resorcinol were identified in 27, 8, 8, 13, 10, and 3 products, respectively.
- No sample contained 4-methoxyphenol or 4-ethoxyphenol.
- From an overview of the 59 whitening products, more than 40% of the products were mislabeled.
- Thirty-four whitening cosmetic products claimed to contain either β -arbutin or α -arbutin. From these products, 50% contained β -arbutin, 29% contained α -arbutin, and 23% products did not contain either arbutin.
- One product contained 3.1% hydroquinone which was 163% of the listed amount on the product label. Hydroquinone in the range of 1.4 – 1.9% was found in four products. Three products were found to contain hydroquinone that was not listed on label.

CONCLUSIONS

- From a commercial point of view, arbutin is becoming a popular whitening agent positively accepted by the consumer as a “natural and safer alternative” to the golden standard, hydroquinone.
- Due to the economical interest in plant containing arbutin, a systematic analysis of plants known for their content of arbutin and related species has been performed. This survey enabled the identification of plants with high content of β -arbutin, which could potentially be added to cosmetic preparations as a natural source of arbutin
- From a toxicological point of view, it is vital to identify the potential risks of generation of hydroquinone upon storage and topical application. The performed stability studies confirmed the instability of arbutin in some cosmetic formulations (especially in liquid preparations and depending on the pH) after long term storage and upon enzymatic conversion. It is relevant to note that arbutin degradation did not necessarily correlate with an increase of hydroquinone content
- A survey of commercial preparations has also been performed in order to identify the most commonly used whitening agents which may possibly become objects of safety investigations in the near future

**Comparative studies on the chemical and enzymatic stability of alpha and beta
arbutin**

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Synopsis

OBJECTIVE: The aim of this study was to establish a comparative analysis of the chemical and enzymatic stability of α - and β -arbutins as potential source of the substance of concern hydroquinone. The study was performed using an array of techniques including HPLC-PDA, nuclear magnetic resonance and optical rotation. Both arbutins are emerging as popular and effective skin whiteners, by acting as tyrosinase inhibitors in a fashion similar to the popular whitening agent hydroquinone. Due to their structural similarity to the regulated agent hydroquinone, both arbutins may be regarded as potential sources of the active aglycone after chemical or metabolic conversion. **METHODS:** Various cosmetic formulations including creams, sera, gels and lotions were analyzed by HPLC-PDA for their arbutin and hydroquinone content in freshly opened and aged samples stored for 16 months. Solutions of pure compounds were also aged and periodically checked for degradation products by using 1D and 2D NMR experiments and optical rotation measurements. The metabolic stability was investigated using pear peels as a biological model. **RESULTS:** Both arbutins were found to be stable in water and methanol solutions in the absence of buffer or stabilizers. Their stability in cosmetic formulations however was found to depend on the type of formulation and pH. Both compounds were unstable under strong hydrolytic conditions, with consequent release of hydroquinone. Enzymatic instability of both arbutins was also observed, although no formation of hydroquinone was observed under the chosen experimental conditions. **CONCLUSION:** Both arbutins were found to possess similar stability profiles, and to be more prone to *in vivo* rather than *in chemico* degradation, although no hydroquinone was found after enzymatic hydrolysis. Also, no epimerization

was observed in any of the tested conditions. Diverse experimental approaches can be applied to analyze the chemical and enzymatic stability of arbutins in regard to the potential release of hydroquinone in different types of preparations. These result showed the potential use of NMR and OR as complementary investigative tools for the stability and safety assessment of arbutin along with more established HPLC methods.

Keywords

Chemical analysis, Formulation/stability, β -Arbutin, α -Arbutin, Hydroquinone, Whitening cosmetics

Introduction

Skin whitening agents are widely used in topical formulations for the treatment of pigmentation disorders, such as melasma, age spots or post-inflammatory hyperpigmentation, or for cosmetic purposes, to lighten or even the skin tone. In the last decade, the demand of skin-whitening products has increased steadily, becoming a multi-billion market, especially in countries where a lighter complexion is strongly associated to the idea of beauty. Among the several chemical agents, hydroquinone (HQ, **3**, Fig. 1S Supporting information) is one of the most prescribed ingredients. Hydroquinone inhibits the melanin synthesis by interfering with the enzymatic activity of tyrosinases, which convert the amino acid tyrosine into melanin [1]. Alpha- and beta-arbutins are glycoside derivatives of HQ, and also became popular skin whitening agents by acting in a similar fashion to their aglycone hydroquinone [2, 3]. β -Arbutin (4-hydroxyphenyl β -D-glucopyranoside, **1**, Fig. 1S) has achieved a positive acceptance among consumers, as a natural and effective alternative to hydroquinone and other toxic whitening agents [4, 5]. β -Arbutin occurs naturally in many plants of the *Ericaceae* and *Caprifoliaceae* families [6-8] and in pears (*Pyrus communis* L.) [9]. Unlike the β -anomer, α -arbutin (4-hydroxyphenyl α -D-glucopyranoside, **2**, Fig. 1S) is most commonly obtained by chemical or biotechnological routes [10].

Stability studies proposed so far mainly focused on the stability of β -arbutin, while less information is available for the α -anomer. Tong, *et al.* [11] assessed the stability of β -arbutin in a range of pH between 4 and 9, using a UV spectrophotometric method (not coupled to HPLC). Liu, *et al.* reported the stability of α -arbutin in different additives used in cosmetics

[12]. In any case, none of the existing methods provided a comparison between the two compounds. Analytical studies on cosmetic formulations are usually performed on creams [13]. Nonetheless, arbutin preparations on the market can be quite diverse in terms of physico-chemical features. Whitening cosmetic formulations range from creams to oil- or water-based gels and sera, to facial sheets, lotions and soaps. The final pH of the formulation and its specific composition, along with the packaging design, can dramatically affect the stability of arbutins by accelerating or slowing the aging process and the potential release of hydroquinone in the preparation. Formulations containing both arbutins can also be found on the market, thus methods to analyze and distinguish arbutins in complex formulations for quality control and stability studies are desirable. The development of traditional HPLC and UPLC methods can be challenging due to the close structural similarity between the two compounds, with elevated risk of co-elution, which can go unnoticed as both compounds possess identical mass and UV properties. Moreover, both arbutins can be regarded as a potential source of HQ, *in vivo* or after chemical decomposition. In the present work, a comparative study on the stability of α - and β -arbutin was undertaken using an array of diverse techniques to discriminate among the two structurally similar compounds. The effect of formulation and pH of commercial cosmetic preparations after 16 months storage was examined by HPLC-PDA. Stability studies have been also performed for both pure compounds under different chemical conditions using various analytical techniques, such as NMR, optical rotation and HPLC-PDA. Both arbutins may also generate hydroquinone *in vivo*, through skin metabolism or exogenous transformation by skin bacteria. For this reason,

enzymatic stability studies have been performed using pear peels as a biological model for *in vitro* studies.

Materials and Methods

Chemicals. α -Arbutin (**2**, CAS n. 84380-01-8) was acquired from Royal DSM (Heerlen, Netherlands). Hydroquinone (**3**, CAS n. 123-31-9) and β -arbutin (**1**, CAS n. 497-76-7) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The identity and purity was assessed using HR-ESI-MS, chromatographic methods and by ^1H and ^{13}C NMR. Pear samples were purchased from a local source (Oxford, MS 38655, USA). The pear variety used in this study was Red d'Anjou. Cosmetic products (including creams, sera, gels and lotions, C1-C9) containing α - or β -arbutin were purchased from the internet.

NMR studies. Four to 7 mg of pure compounds or mixtures was dissolved in 200 μL of deuterated solvent. ^1H and ^{13}C spectra of the samples were recorded to provide a reference standard. Three mm NMR tubes were used and the samples stored in the corresponding deuterated solvent at room temperature under artificial light for 8 h/day for 12 months. ^1H and ^{13}C spectra were periodically collected in the same conditions as for the standard compound. ^1H (500 MHz) and ^{13}C (126 MHz) NMR spectra were recorded on Agilent DD2-500 NMR spectrometers equipped with an oneNMR probe and Agilent Vnmrj 3.2 software. Chemical shifts were referenced to the residual solvent signal (CD_3OD : δ_{H} 3.31 ppm, δ_{C} 49.0 ppm, D_2O : δ_{H} 4.79 ppm). ^1H and ^{13}C -NMR experiments were performed at 25 $^\circ\text{C}$ (^1H parameters: resolution = 0.23 Hz, nt = 8, at = 5, d1 = 10 s, sw = 7485.0; ^{13}C parameters: nt=1000, d1= 3.5 s, sw = 31250.0). One-bond heteronuclear ^1H - ^{13}C correlation experiments were performed using gHSQCAD experiments (nt = 8, ni= 256).

Specific rotation. The samples to be analyzed for rotatory power were prepared in methanol or water (HPLC grade) according to literature data [14-16]. Solutions of α -arbutin were prepared to final concentrations of 2 g/100 mL in H₂O and 1.23 g/100 mL in MeOH; β -arbutin was diluted to final concentrations of 2 g/100 mL in H₂O and 0.56 g/100 mL in MeOH, respectively. The solutions were kept for a year under artificial light at room temperature for at least 8 h/day and specific rotatory power values were periodically acquired in the same standard conditions. A Rudolph Research Analytical digital polarimeter was used for the measure of the rotatory power. The readings were performed at 589 nm and 25 °C (corrected to 20 °C), using a 25 mm path length microcell.

HPLC-PDA analysis. The analytical investigations were performed on a Waters Alliance 2695 HPLC system using a data station with a Waters Empower 2 software. A Synergi Hydro RP column (250 \times 4.6 mm; 4 μ m) from Phenomenex was used, and the temperature was maintained at 45 °C. The column was equipped with a 2 cm LC-18 guard column (Phenomenex). The mobile phase consisted of water (A) and methanol (B) with both solvents containing 0.1% acetic acid. The analyses were performed according to a previous method [17]. The detection wavelength was 280 nm for both arbutins and hydroquinone.

Extraction solution and standard preparation for HPLC analysis. The extraction solution used for the sample preparation of standards, cosmetics and pear samples was obtained by mixing 10% MeOH in 20 mM NaH₂PO₄ buffer (v/v, pH 2.3). An individual stock solution of standard compounds **1-3** were prepared at a concentration of 5.0 mg/mL in the extraction solvent.

Chemical hydrolysis. 0.2 Milligram of β -arbutin or α -arbutin was accurately weighed and

dissolved individually in 1.0 mL 10% MeOH in acid condition (pH 1.1). The sample was heated in boiling water for 2.5 h, then analyzed by HPLC-PDA.

Investigation of skin whitening formulations. The cosmetics used were creams, sera, lotions and gels. The pH of the various cosmetic preparation was measured according to the European guidelines [18]. A 10% solution or dispersion of the product was prepared by dissolving 1 g of cream in 10 g of HPLC grade water. The pH values were measured in triplicates and the results were averaged. A pH meter (Model IQ125 Professional, IQ Scientific Instrument, Inc., Carlsbad, CA, USA) was used and calibrated with reference solutions pH 4.0, 7.0 and 10.0 before the measurements.

The skin whitening samples were prepared for HPLC analysis as follows. Five hundred milligram of each product was accurately weighted into a 15 mL centrifuge tube and 8 mL of the extraction solution was added. The samples were vortexed and sonicated for 30 minutes. After sonication, the samples were centrifuged at 4000 rpm for 30 min and the supernatant was transferred into a 10 mL volumetric flask. Two mL of the extraction solution was added into the centrifuge tube and sonicated again for 30 min. After centrifugation, the supernatants were combined, and the volume was adjusted to 10 mL with the extraction solution. The extracts were mixed thoroughly, and filtered with 0.45 μ m PTFE filter prior to HPLC analysis.

Spiking and extraction of pear samples. The epicarp of 12 fresh pears was ground after separation from the pulp. Two grams of the ground sample was accurately weighed into a 15 mL centrifuge tube and extracted by sonication using 3 mL of extraction solvent. The samples were centrifuged at 4000 rpm for 15 min and the supernatant was transferred into a

10 mL volumetric flask and the procedure repeated 2 more times. After centrifugation, the supernatants were combined, and the volume was adjusted to 10 mL with the extraction solution. The extracts were mixed thoroughly, and filtered with 0.45 μ m PTFE filter prior to HPLC analysis. Spiked samples were obtained by adding 100 μ L of a solution of either α - or β -arbutin (10 mg/mL H₂O).

The spiked samples were incubated at room temperature for 0, ½, 1, 2, 4 and 8 h respectively. After incubation, the enzymatic activity was quenched by addition of extraction solution (3 mL) and extracted as explained for the fresh pears samples.

Control samples were prepared following the procedures by pre-treating the plant material to deactivate the enzymes, removing the β -arbutin present in the fresh peels, and spiking a known amount of β - or α -arbutin standard compounds. Forty grams of fresh ground peels were accurately weighed and split in four 50 mL centrifuge tubes. The samples were extracted with methanol (45 mL) and centrifuged at 4000 rpm for 30 min. The procedure was repeated four times then the sample were extracted with water (50 mL) and centrifuged for two more times to remove the excess of methanol. The remaining solid material was weighed and adjusted with water to restore the initial amount of water content. Two grams of the obtained sample was then accurately weighed in a 15 mL centrifuge tube, the samples were spiked with β - or α -arbutin and extracted as explained above.

Results and Discussion.

Stability of arbutins in cosmetic formulations.

In order to investigate the effect of pH and formulation on the stability of **1** and **2** in cosmetic preparations, nine types of products were analyzed. Two samples were found to have pH values above 8 and one less than 4 (Table I). The samples were all stored at room temperature for 16 months to mimic the typical storage conditions used by the consumer, then re-analyzed by HPLC-PDA under the same conditions as the freshly opened samples. Five samples out of 9 were found to contain above 95% of the initially determined α - or β -arbutin content. All the sera samples (C3, C5, C9) showed losses of arbutin content between 5.8 and 20.7%. Recovery within experimental error was found for creams, lotions and gel formulations, with the exception of sample C2, which was also determined to have a pH value of 3.3. As summarized in Table I, pH values above 4 didn't affect the stability of either arbutin in non-liquid preparations (C1, C4, C6, C7, and C8) under the tested conditions, even after 16 months. On the contrary, the nature of the formulation may affect the stability of compounds **1** and **2**. Both arbutins were found to be less stable in liquid preparations, with a major loss in the presence of basic solutions, such as sample C5. β -Arbutin is reported to be a highly photosensitive compound [19], and sunscreen agents are often added in whitening cosmetics to prevent β -arbutin degradation. After UV exposure, the pH conditions may affect the stability of the compounds, and accelerate the decomposition rate. Several stability studies have been reported in the literature for β -arbutin, with differing outcomes [20, 21]. Our results confirmed that both arbutins may be unstable with dependence on the pH and

type of formulations, thus both factor have to be accounted for in the design of new formulations.

Chemical stability studies.

Based on the results obtained from long term storage of different whitening formulations, additional studies on the stability of pure compounds in water or alcoholic (methanol) solutions were performed in the absence of acid or bases to verify if the instability of arbutin may have been related to a photo-catalyzed degradation independent from the pH conditions. These studies have been performed using Nuclear Magnetic Resonance (NMR) or optical rotation (OR) analysis. The samples were stored in NMR tubes or clear scintillation vials at room temperature under normal light irradiation for at least 8 h/day. Although less sensitive than HPLC, NMR can provide very useful molecular insights, especially when comparing structurally similar compounds, such as α - and β -arbutin, containing identical functional groups but different structural orientation. The NMR spectra of **1** and **2** possess unique ^1H and ^{13}C features at the anomeric position. The anomeric proton signal of β -arbutin at 500 MHz can be identified as a doublet at δ 4.74 in CD_3OD (Fig. 2Sa, Supporting Information), or at δ 4.96 in D_2O (Fig. 3Sa), while for α -arbutin, the same signal can be observed at δ 5.29 in CD_3OD or δ 5.46 in D_2O , respectively (Figs. 2Sd and 3Sd). The coupling constants for the anomeric proton were found to be $J = 7.3$ Hz for the β - and $J = 3.7$ Hz for the α -anomer. These values are in agreement with a predicted diaxial coupling ($J = 7\text{--}9$ Hz) and axial-equatorial coupling ($J = 2\text{--}4$ Hz) which are associated to a β - and α -configuration, respectively [22]. The ^{13}C NMR spectra of the two compounds are also very similar, with

the exception of the C1' position, which is also affected by the sugar configuration [23]. The observed chemical shifts at 500 MHz were 103.2 ppm and 103.9 ppm (D₂O, Fig. 5Sa) in the case of β -arbutin and δ 100.2 (CD₃OD, Fig. 4Sd) and δ 100.4 (in D₂O, Fig. 5Sd) for α -arbutin. The samples of pure α - or β -arbutin were stored in NMR tubes and analyzed at different time intervals (see Figs. 2-5S). If hydrolysis resulting in the generation of hydroquinone and D-glucose occurred, the presence of a new ¹³C signal at 96 ppm would be expected. No signals at 96 ppm were detected for either arbutin, thus confirming the lack of hydrolysis events in all the analyzed samples, even after 12 months. In order to confirm the finding from 1D ¹H experiments, all the samples were also analyzed using 2D hetero-correlation analysis, where the anomeric signals can be clearly identified without residual solvent interference. The results from gHSQCAD analysis are presented in Fig. 6S and 7S. The 2D NMR experiments confirmed the absence of release of free glucose or anomerization processes in the analyzed samples.

In a similar fashion to the NMR study, sample solutions were analyzed for changes of the rotatory power in non-deuterated methanol and water at the same conditions reported in the literature [15, 16, 24] (Table IS, Supporting Information). The measured OR values were stable within the precision of the instrument for six months. The results from both NMR and optical rotation experiments with solutions of pure standard compounds supported the statement from the opinion of the European Commission on β -arbutin, which reports the compound as “stable under sunlight when kept at 3% solution in ethanol in transparent glass bottle for 30 days” [25]. Similar results were also obtained for α -arbutin. Minor degradation peaks (below the LOQ of the detection method) in ¹H NMR spectra were observed after 12

months, but none of these signals have been correlated to the formation of glucose as a result of hydrolysis. Also, no epimerization was observed under any of the experimental conditions tested. Both NMR and optical rotation results didn't indicate the presence hydroquinone as a degradation by-product.

To verify that hydroquinone can be chemically obtained as degradation product of arbutins, a standard chemical hydrolysis procedure [26] was also performed and the HPLC results are shown in Fig. 1. Both arbutins were found to be readily hydrolyzed in 2.5 h under strong acidic (pH 1.1) and temperature conditions, with a complete loss of arbutin and generation of hydroquinone as major degradation product. The yields of hydroquinone converted from β - and α -arbutin were 84.9 and 89.6%, respectively.

Enzymatic stability studies.

The accumulation of β -arbutin in pear tissues is enzymatically controlled [27, 28], an observation which has been related to the potential role of β -arbutin in the defense mechanism from pathogens like fireblight [29-33]. Pear peels are known for their content of β -glucosidases and peroxidases as major enzymes involved in the arbutin metabolism [29, 34]. Peroxidases are also involved in melanogenesis and human skin metabolism [35]. The fates of both **1** and **2** *in vivo* were thus determined using pear peels as surrogate *in vivo* models. The concentrations of both arbutins and hydroquinone were evaluated by HPLC-PDA. The initial content of β -arbutin present in pear peels was estimated to be 0.138 mg/g in freshly prepared samples. The concentration of β -arbutin rapidly decreased in less than 8 h, with a total loss of > 90% after 24 h (Fig. 2a). The peel samples were then spiked with

269 either β - (Fig. 2b) or α -arbutin 0.5 mg/g (Fig. 2c). After 8 h, a loss of 62.4 and 49.4% of α -
270 and β -arbutin, respectively, were measured. In the control samples, which were pre-treated
271 in MeOH to quench the enzymatic activity, the loss of arbutins was negligible even after 24
272 h, thus excluding the possible role of external factors (e.g. light, air exposure, heat) in the
273 experimentally observed degradation processes. From the PDA UV profile (Fig. 8S,
274 Supporting Information), no hydroquinone was generated during the decomposition of either
275 arbutin. Any peak at the retention time of hydroquinone (12.57 min) was below the limit of
276 detection even after 24 h. β -Arbutin is known to hydrolyze enzymatically to hydroquinone,
277 and some of the biological and toxicological properties of β -arbutin can be directly related
278 to its aglycone after *in vivo* metabolism [36, 37]. Bang *et al.* reported the potential release of
279 hydroquinone *in vivo* through enzymatic hydrolysis of β -arbutin by skin microflora [38].
280 However, the results herein presented demonstrated that arbutin metabolism may not
281 generate hydroquinone as unique by-product. The authors hypothesize that hydroquinone
282 may be formed after enzymatic hydrolysis but not detectable due to the chemical or
283 enzymatic instability of the compound in the chosen experimental conditions. Short term
284 studies on solutions of hydroquinone at different pH conditions demonstrated that the
285 compound partially degrades at pH>5, with a loss of 20% of hydroquinone at pH 7.6 after
286 24 h (data not shown), thus the compound may be generated *in vivo* and rapidly degrade due
287 to the chemical conditions of the cellular environment. Another hypothesis is derived from
288 the observation that both hydroquinone and arbutin are potential substrate of peroxidases
289 [34, 39]. It is thus possible that either hydroquinone is not released in *in vivo* conditions or

it is rapidly converted into more stable end-products. Further studies will be necessary to confirm whether the observed results using pear peels can be translated to skin metabolism.

Conclusions

The long term stability of arbutins in cosmetic formulations stored for 16 months was found variable depending on the formulation and pH, with a partial loss in basic liquid preparations. Both arbutins were found to be stable in neutral solutions (in the absence of stabilizers or buffers). On the other hand, both **1** and **2** were found to be unstable under strong hydrolytic conditions with consequent formation of hydroquinone. Metabolic instability of both arbutins also need to be considered as a potential concern, although the potential release or accumulation of hydroquinone in the skin after topical application of arbutins may not necessarily occur.

303

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310

311 **Conflicts of Interest**

312 The authors declare no conflict of interest.

313

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Figure Legends

Fig. 1 HPLC-PDA chromatograms of β -arbutin, α -arbutin, and related products after hydrolysis (**1**, β -arbutin; **2**, α -arbutin; **3**, hydroquinone)

Fig. 2 Stability studies results using fresh pear peels. (—) Control (- - -) reaction A) degradation kinetic of β -arbutin naturally occurring in the fresh peels, B) Sample spiked with 0.5 mg/g of β -arbutin, C) degradation kinetic of α -arbutin in samples spiked with 0.5 mg/g compound.

Table I. Stability study on commercial whitening formulations.

			β -Arbutin			α -Arbutin		
CODE	Formulation	pH	In. amount (mg/100 mg)	16 month (mg/100 mg)	Recover (%)	In. amount (mg/100 mg)	16 month (mg/100 mg)	Recover (%)
C1	cream	8.5	0	0	-	1.68	1.76	105.3
C2	cream	3.3	3.20	2.95	92.2	0.00	0.00	-
C3	serum	7	0.00	0.00	-	2.10	1.94	92.4
C4	cream	7.6	0.00	0.00	-	0.15	0.16	106.7
C5	serum	8.8	0.21	0.17	79.3	0.00	0.00	-
C6	gel	4.4	0.13	0.13	100.0	0.00	0.00	-
C7	cream	7.7	0.00	0.00	-	1.01	1.03	102.0
C8	lotion	6.7	3.10	3.05	98.5	0	0	-
C9	serum	4.3	4.30	4.05	94.2	0	0	-

Table I.

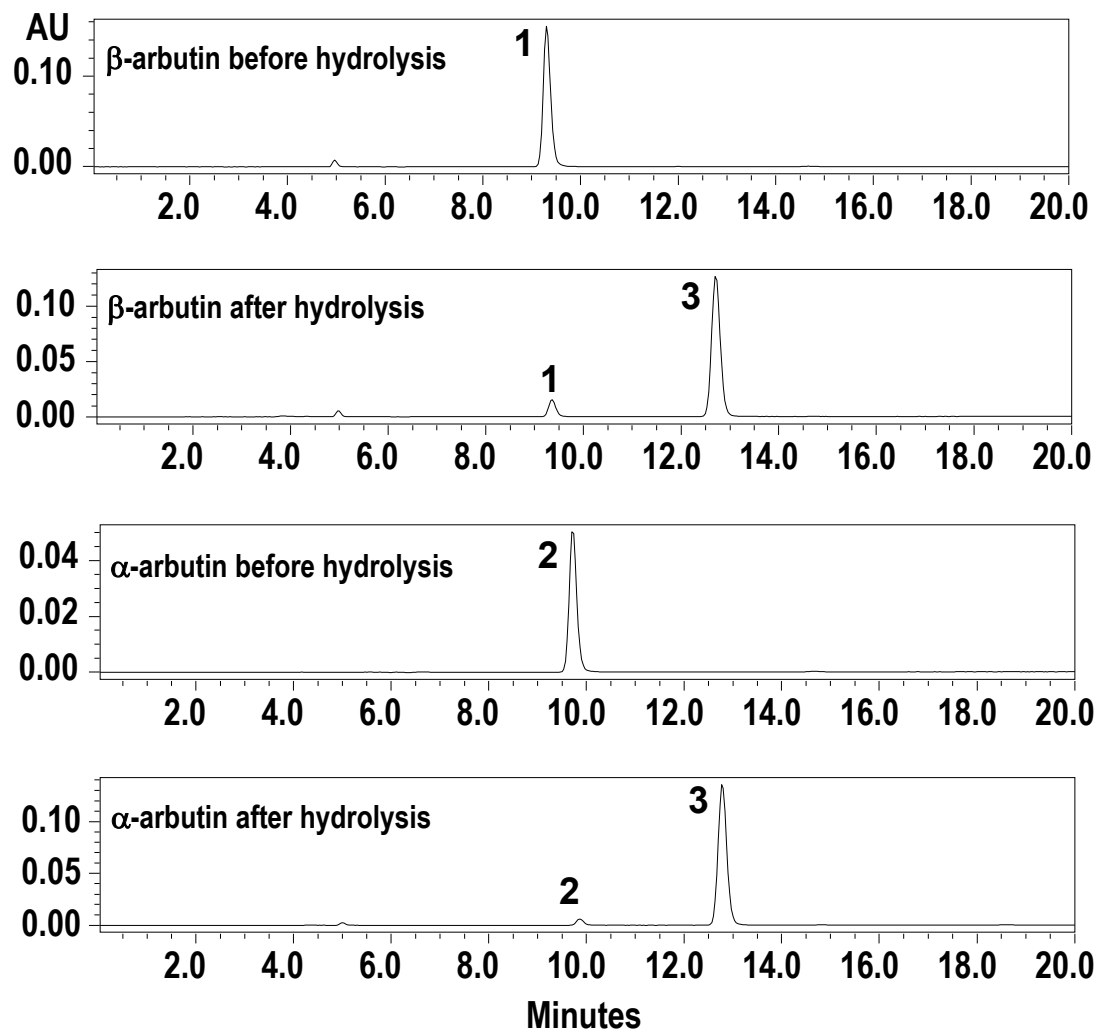


Figure 1

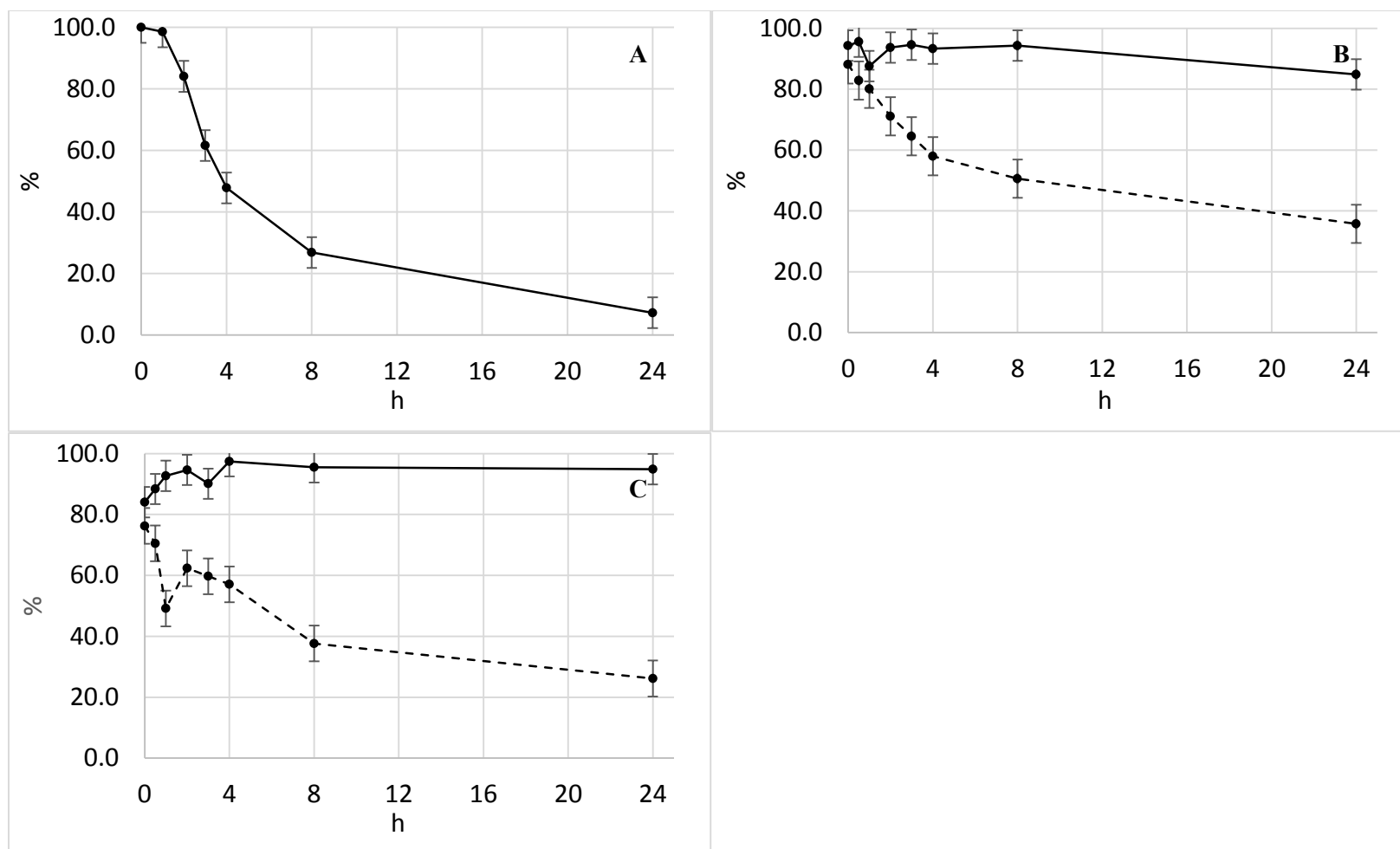


Figure 2

From: [Ikhlas Khan](#)
To: [Hansen, Patricia A](#)
Subject: Re: list
Date: Tuesday, June 30, 2015 7:35:10 PM

Thanks for the reply, please let me know if I can be any assistance to you.

IK

From: <Hansen>, "patricia.hansen@fda.hhs.gov" <patricia.hansen@fda.hhs.gov>
Date: Tuesday, June 30, 2015 at 5:07 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: list

Hi, Ikhlas. I hope your trip went smoothly and the visit was a good one. Glad to hear you received the list. The new job is very busy/hectic but also very interesting and a good opportunity. Take care.
PH

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Monday, June 29, 2015 5:57 PM
To: Hansen, Patricia A
Cc: Katz, Linda
Subject: Re: list

Dear Pat

I just got back from India. It was short trip to visit my Mom. I did the list from Linda and the group. Once we provide all the information I assume it will be followed by conference call.

How are you doing at new job.

IK

From: "patricia.hansen@fda.hhs.gov" <patricia.hansen@fda.hhs.gov>
Date: Wednesday, June 17, 2015 at 12:21 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Subject: RE: list

Hi, Ikhlas. Sorry not to get back to you sooner.

I think the conversations we had while on our visit were very helpful.

By copy of this note, I'll alert Linda to your request for the fragrance allergen list. We talked about it during our visit and she may have assigned it to someone already, but I'm not sure.

Is there a conference call already scheduled, perhaps with the dietary supplement group and others or are you talking about something else?

Hope all is well.

PH

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Friday, June 05, 2015 10:58 AM

To: Hansen, Patricia A

Subject: list

Hi Pat

I know this is your first week and must be busy. Could you please share the list of 26 and may be 80 if possible.

It wil help to us to see what is coming but you will still have time to prioritize before we have conference call

Thanks

IK

From: [Ikhlas Khan](#)
To: [Hansen, Patricia A](#)
Subject: how are you
Date: Friday, July 10, 2015 11:14:40 AM

Hi Pat

I was asked by Linda to provide material which I just did. You were not on email list so I BCC you. I am not sure you would like to be included or not. Looks like Nalissa will be incharge and I want to be sure that our collaboration remain smooth.

If your time allows we can talk on phone, you have my cell# (b) (6)

IK

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: <no subject>
Date: Monday, June 16, 2014 9:42:49 AM
Importance: High

Hi Rahul

I hope you reached safely. I need your assistance, My Niece qualified for GPAT and now appeared in NIPER test. Now they do NIPER test it means she can get admission in any NIPER and it does not have to be Mohali. I sent an email to Bhutani but no response. Do you still have some connection there.

Her application# (b) (6)

Registration# (b) (6)

Her name is (b) (6)

IK

From: SATYANARAYANARAJU SAGI
To: [Pawar, Rahul](#)
Subject: RE: Postdoc
Date: Friday, July 22, 2016 9:02:05 AM

Hi Rahul,

I posted the documents through USPS Priority service and the tracking shows it delivered on 18th July.

Address posted to

Detra Brown

Management Analyst

Food and Drug Administration Center for Food Safety and Applied Nutrition

Office of Regulatory Science

5100 Paint Branch Parkway

College Park, MD 20740

Thanks

Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Friday, July 22, 2016 6:53 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Postdoc

Raju, how did you send the documents to Detra?

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Thursday, July 21, 2016 2:39 PM
To: Pawar, Rahul
Subject: Re: Postdoc

Ok Rahul,

Thanks

Raju

From: Pawar, Rahul <Rahul.Pawar@fda.hhs.gov>
Sent: Thursday, July 21, 2016 1:34:07 PM
To: SATYANARAYANARAJU SAGI
Subject: RE: Postdoc

Not yet..

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Thursday, July 21, 2016 2:30 PM
To: Pawar, Rahul
Subject: Re: Postdoc

Hi Rahul,
Do you hear any updates on my application??

Thanks
Raju

Satyanarayanaraju Sagi
Post Doctoral Research Associate
TCRC Q305
National Center for Natural Products Research
School of Pharmacy
University of Mississippi
Univeristy, MS 38677
Phone: 662 915 7610
Email: ssagi@olemiss.edu

From: Pawar, Rahul <Rahul.Pawar@fda.hhs.gov>
Sent: Tuesday, July 12, 2016 2:12 PM
To: SATYANARAYANARAJU SAGI
Subject: RE: Postdoc

Great!

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Tuesday, July 12, 2016 3:12 PM
To: Pawar, Rahul
Subject: RE: Postdoc

Posted required documents to Detra Brown today.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Wednesday, July 06, 2016 9:18 PM
To: SATYANARAYANARAJU SAGI
Subject: RE: Postdoc

E-arive looks ok, I will probably use only one email address to avoid confusion.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Wednesday, July 06, 2016 3:35 PM
To: Pawar, Rahul
Subject: RE: Postdoc

Hi Rahul,

Please find the attachments of ORISE application and eArrive form.

I filled the details I knew please check these forms and let me know if these are ok.

Dr. Khan is out of office, I will get the reference letter from him and send all the enclosure to Detra Brown.

Thanks

Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]

Sent: Tuesday, June 28, 2016 12:35 PM

To: SATYANARAYANARAJU SAGI

Cc: Brown, Detra

Subject: FW: Postdoc

Hi Raju,

The attached ORISE application and eArrive form (Personal, Contact Information, Personal Work Authorization, if applicable, and Federal Work sections) need to be completed and returned with the following documents:

Resume/CV

College Transcripts (Official)

Letters of Reference (2)

Proof of Health Insurance

Send the package to Detra Brown. Let me know if I can assist further.

Detra Brown

Management Analyst

Food and Drug Administration Center for Food Safety and Applied Nutrition

Office of Regulatory Science

5100 Paint Branch Parkway

College Park, MD 20740

240-402-3004 (P)

301-436-2332 (F)

Detra.Brown@fda.hhs.gov

Thanks

Rahul

From: Noonan, Gregory

Sent: Monday, June 27, 2016 3:54 PM

To: Brown, Detra
Cc: Pawar, Rahul
Subject: Postdoc

We have found a person to select for the dietary supplement ORISE project. Can you send Rahul the necessary paperwork so we can get the process underway?

Thanks

Greg

(b) (6)

From: [Pawar, Rahul](#)
To: [Brown, Detra](#)
Subject: RE: Postdoc
Date: Friday, July 22, 2016 7:54:00 AM

Ok, I will check with him and let you know.

From: Brown, Detra
Sent: Friday, July 22, 2016 7:53 AM
To: Pawar, Rahul
Subject: RE: Postdoc

I have checked, I do not have anything from him in my file or email.

From: Pawar, Rahul
Sent: Friday, July 22, 2016 7:53 AM
To: Brown, Detra
Subject: RE: Postdoc

Thanks Detra,

I am worried. This is what he communicated with me on July 12th "Posted required documents to Detra Brown today."

This means you did not received them? Can you please check?

Thanks

Rahul

From: Brown, Detra
Sent: Friday, July 22, 2016 7:22 AM
To: Pawar, Rahul
Subject: FW: Postdoc

Rahul,

This is the last communication that I have with Dr. Sagi, he never responded to me with the information requested below.

Thanks

*Detra Brown
Management Analyst
Food and Drug Administration
Center for Food Safety
and Applied Nutrition
Office of Regulatory Science
5001 Campus Drive
College Park, MD 20740*

240-402-3004 (P)

301-436-2332 (F)

From: Pawar, Rahul
Sent: Tuesday, June 28, 2016 1:35 PM
To: SATYANARAYANARAJU SAGI (ssagi@olemiss.edu)
Cc: Brown, Detra
Subject: FW: Postdoc

Hi Raju,

The attached ORISE application and eArrive form (Personal, Contact Information, Personal Work Authorization, if applicable, and Federal Work sections) need to be completed and returned with the following documents:

Resume/CV
College Transcripts (Official)
Letters of Reference (2)
Proof of Health Insurance

Send the package to Detra Brown. Let me know if I can assist further.

Detra Brown
Management Analyst
Food and Drug Administration Center for Food Safety and Applied Nutrition
Office of Regulatory Science
5100 Paint Branch Parkway
College Park, MD 20740
240-402-3004 (P)
301-436-2332 (F)
Detra.Brown@fda.hhs.gov

Thanks
Rahul

From: Noonan, Gregory
Sent: Monday, June 27, 2016 3:54 PM
To: Brown, Detra
Cc: Pawar, Rahul
Subject: Postdoc

We have found a person to select for the dietary supplement ORISE project. Can you send

Rahul the necessary paperwork so we can get the process underway?
Thanks

Greg

(b) (6)

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: <no subject>
Date: Monday, February 11, 2013 3:14:44 PM

Hi Rahul

All your bosses are useless. Can you send me Musser's full designation.

Hope you all doing fine.

Ik

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: <no subject>
Date: Thursday, January 3, 2013 11:59:15 AM

HappyY New Year and the best wished for 2013.
IK

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: <no subject>
Date: Wednesday, August 22, 2012 9:54:29 PM

Sad News, Valli passed away. Her brother will arrange funeral.
ik

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: <no subject>
Date: Tuesday, August 21, 2012 11:07:12 PM
Attachments: [J.Guizhou Inst Tech 1996_1.pdf](#)

Thanks, I will be coming to attend ceremony and congratulations to you too. I should be arriving on 6th evening and will leave on 7th in the evening.
IK

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: <no subject>
Date: Tuesday, May 8, 2012 5:56:23 PM

Dear Rahul

How are you. I know you are working on *Acacia rigidula* and found biogenic amines. I have been asked by Fabricant to do animal behavioral studies on it. Just taking extract of acacia might not help since they are minor compounds. I was wondering if you can provide extract enriched with biogenic amines we can do animal study.

Let me know what you think before you or I talk to Jeanne. Call me if you can
IK

From: Jennifer S. Taylor
To: [Pawar, Rahul](#)
Subject: 2016 ICSB
Date: Thursday, March 31, 2016 5:31:54 PM

Rahul, we are looking forward to seeing you in a couple weeks. If you have finalized your itinerary please send it to me and also let me know if you will need the shuttle service. Let me know if you need any assistance in your preparations.

Sincerely,

Jennifer Taylor

Jennifer Taylor
Program Coordinator
University of Mississippi
National Center for Natural Products Research
3012 Thad Cochran
P.O. Box 1848
University, MS 38677
✉ jnnfrtyl@olemiss.edu
☎ 662-915-1090
📠 662-915-7989

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From: ICSB Web Team
To: [Pawar, Rahul](#)
Subject: A Happy Thanksgiving and a Reminder from the ICSB Team!
Date: Monday, November 24, 2014 4:31:41 PM

ICSB Header Generic



15th Annual Oxford International Conference on the Science of Botanicals

The ICSB Web Team would like to wish you a very happy Thanksgiving Holiday, and remind you of the important deadlines for the upcoming Oxford International Conference on the Science of Botanicals, to be held April 13th to the 16th, 2015.

Firstly, we would like to announce that the confirmed list of invited speakers is now available on our web site at <http://oxfordicsb.org/agenda>. We have lined up an exciting array of speakers from all over the world and from all parts of natural products science, research and regulation.

December 15th, 2014 is the final deadline for oral and poster abstracts. All abstracts for this year must be submitted through our online portal at <http://abstracts.oxfordicsb.org>. Abstract formatting guidelines and instructions are available within the portal, but don't hesitate to contact us at icsb@olemiss.edu if you have any questions about or issues with the site.

Don't forget to register as soon as possible! Rates will go up closer to conference time, and the sooner you register, the sooner you can complete your travel plans! You can register online [via our CVent page](#). As always, feel free to direct any questions you may have to us at ICSB@olemiss.edu, and don't forget to visit our site for further info, and follow us Facebook for up-to-the-minute announcements and updates ([Facebook.com/OxfordICSB](https://www.facebook.com/OxfordICSB)).

We look forward to seeing you all at the 15th Annual ICSB, and we look forward to reviewing all your abstract submissions!

ICSB Web Team,
<http://oxfordicsb.org>
<http://facebook.com/OxfordICSB>
icsb@olemiss.edu

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If you no longer want to receive emails from Micah Quinn please click the link below.

[Opt-Out](#)

From: YANHONG WANG
To: [Pawar, Rahul](#)
Subject: about FDA position
Date: Friday, May 11, 2012 11:05:50 AM

Dear Rahul,

I didn't see you on this year's FDA conference. How is your everything going?

Dr. Khan told me that FDA had an open position for instrumental analysis on natural products, and suggested me to have your help. Would you please provide me more information on this position? If I want to apply for it, how should I prepare for it? Thank you very much.

Best regards,

Yan-Hong Wang

From: [Pawar, Rahul](#)
To: ikhan@olemiss.edu
Subject: Acacai paper
Date: Wednesday, October 30, 2013 9:10:00 AM
Attachments: [Pawar Acacia JPBA2014.pdf](#)

Good morning Sir,
The acacia paper got published yesterday, Sending you a copy.
Thank you
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: ikhan@olemiss.edu
Subject: Acacia news
Date: Thursday, November 21, 2013 3:43:00 PM

Hello Sir,
FYI

<http://www.foxnews.com/health/2013/11/21/some-weight-loss-supplements-contain-amphetamine-like-compound/>

<http://www.usatoday.com/story/news/nation/2013/11/18/fda-scientists-find-amphetamine-like-compound-in-dietary-supplements/3627963/>

Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: TROY J SMILLIE
To: [Pawar, Rahul](#)
Subject: Acacia
Date: Thursday, September 26, 2013 10:24:46 AM

Dear Rahul,

A while back you had sent us some bulk *Acacia rugidula* material. Do you have the full collection information for this this samples.

Troy Smillie, Ph.D.
Senior Research Scientist
Thad Cochran National Center for Natural Products Research
School of Pharmacy
University of Mississippi
University, MS 38677
Phone 662-915-1168
Fax 662-915-7062
<http://www.olemiss.edu/depts/pharmacy/php/sopquery3.php?id=69>

From: [Pawar, Rahul](#)
To: ikhan@olemiss.edu
Subject: Acaia rigidula
Date: Tuesday, April 7, 2015 10:29:00 AM

FYI- Acacia in news again.

<https://www.pharmamedtechbi.com/publications/the-tan-sheet/23/15/bmpea-in-supplements-spells-trouble-to-researchers>
<http://www.reuters.com/article/2015/04/07/health-dietarysupplements-stimulant-idUSL2N0X400U20150407>

Rahul

Rahul Pawar, Ph.D.
Office of Regulatory Science
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-3077

From: [Pawar, Rahul *](#)
To: ["tsmillie@olemiss.edu"](mailto:tsmillie@olemiss.edu)
Subject: Address
Date: Friday, September 2, 2011 3:09:00 PM

Hi Troy,
My address is

Rahul Pawar
Division of Bioanalytical Chemistry
CFSAN/FDA
5100 Paint Branch Parkway (HFS-717)
College Park, MD, 20740.

Thank you and have a good weekend

Rahul

Rahul Pawar Ph.D.

CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: ICSB Web Team
To: [Pawar, Rahul](#)
Subject: Agenda for the 16th ICSB/ASP now available.
Date: Wednesday, February 24, 2016 9:35:32 AM

ICSB Header Generic



Joint Meeting with 16th Annual International Conference on the Science of Botanicals (ICSB) & 5th Interim American Society of Pharmacognosy (ASP)

Greetings from the ICSB Web Team!

We would like to announce that the agenda for the 16th ICSB and 5th interim ASP meeting is available for viewing and download now at <http://oxfordicsb.org/index.php/agenda>. Poster guidelines are also available now at <http://oxfordicsb.org/index.php/submissions> so be sure to take a look at those if you have submitted a poster this year.

Also, don't forget to register as soon as possible! Rates go up sharply for on-site registration, so be sure to book online. You can register [via our Cvent page](#). As always, feel free to direct any questions you may have to us at ICSB@olemiss.edu, and don't forget to visit our site for further info, and follow us Facebook for up-to-the-minute announcements and updates ([Facebook.com/OxfordICSB](https://www.facebook.com/OxfordICSB)).

Thank you,

ICSB Web Team,
<http://oxfordicsb.org>
<http://facebook.com/OxfordICSB>
icsb@olemiss.edu

Invitation Bottom Banner



If you no longer want to receive emails from Micah Quinn please click the link below.

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From: SATYANARAYANARAJU SAGI
To: [Pawar, Rahul](#)
Cc: [Ikhlas Khan](#)
Subject: Application for research position in your group
Date: Friday, August 21, 2015 2:22:19 PM
Attachments: [Sagi CV-08202015.doc](#)

Dear Dr. Rahul,

I Satyanarayanaraju Sagi, introduce myself as a Post Doctoral Research Associate, working with Dr. Ikhlas Khan, Associate Director, NCNPR, University of Mississippi, Univeristy, Mississippi, USA. During my post doctoral research program, I have been working in analytical department which mainly focus on development of chemical fingerprint profiles of plant extracts using HPTLC, HPLC, UHPLC and UHPLC-MS/MS, the major applicability of these methods are in analyzing dietary supplements claiming to specific plant extracts.

My doctoral work was carried under the guidance of Dr. R. Nageswara Rao, Chief Scientist in Analytical chemistry division. My doctoral thesis mainly focused on bioanalytical method development and validation of anticancer and antihypersensitive drugs. The methods include novel blood collection technique like dried blood spots and ionic liquid based liquid-liquid microextraction from rat serum. My research work also include method of reverse phase chiral LC-MS for chiral separation and in-vitro disposition of chiral enantiomers. During this period, as an active member in natural product group my contribution involves method development for preparative isolation of bioactive from the plant extracts and development fingerprinting profile, quantification of marker compounds in various medicinal plants. I will be helping the research scientist in structure elucidation of new isolates through fragmentation studies on mass spectrometry and conformation by high resolution mass spectrometric studies.

Having more than eight years of experience in the field of analytical chemistry with various expertise's I am keen in exploring an opportunity to work in the area of natural products and dietary supplements, which unambiguously would provide me an opportunity to further improve my knowledge and skills. Considering the interest to reach new heights in the field of analytical chemistry/dietary supplements and with all confidence that I shall be a potential competitor for Research Position in your research group, herewith I am enclosing my curriculum vitae for your kind perusal.

Hope my experience and skills meet your requirements. I feel privileged to furnish more information if you need.

Thank you for your consideration and I hope to hear from you in the near future.

Sagi

Satyanarayanaraju Sagi
Post Doctoral Research Associate
National Center for Natural Products Research
School of Pharmacy
The University of Mississippi

University, MS 38677

Work: 662 915 7610

Email: ssagi@olemiss.edu;

(b) (6)

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: articles
Date: Monday, May 4, 2015 11:12:30 AM

could you please mail me the following:

1. AOCS (1999) Official method Ce 1h-05. In: Firestone D (ed) Official methods and recommended practices of the AOCS, 5th edn. AOCS, Champaign, IL
2. AOAC Int (2001) Official method of analysis 996.06, revised 2001. In: Official methods of analysis, 18th edn. AOAC International, Gaithersburg, MD

thanks
bharathi

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Automatic reply:
Date: Wednesday, September 5, 2012 2:33:17 PM

Dr. Khan is out of office until September 10th. If you need assistance please contact Ms. Jennifer S. Taylor <jnnfrtyl@olemiss.edu> tel# 662 915 1090 or Dr. Troy Smillie tsmillie@olemiss.edu

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Automatic reply: Dietary supplements labeled as blood sugar management (purchased)
Date: Thursday, August 27, 2015 11:32:43 AM

Dr. Khan is out of office till September 10, 2015. Please contact Ms. Jennifer jnnfrtyl@olemiss.edu or by phone 662 915 1090 if you need immediate assistance.

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Automatic reply: DMAA paper
Date: Friday, February 14, 2014 3:09:12 PM

Dr. Khan is out of office till Feb 26th. Please contact Ms. Jennifer Taylor if you need assistance Jennifer Taylor <jnnfrtyl@olemiss.edu>, tel# 662 915 1090.

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Automatic reply: Hello
Date: Wednesday, February 11, 2015 4:24:40 PM

Dr Khan is out of office till Feb 25th. If you need assistance please contact Ms Jennifer jnnfrtyl@olemiss.edu or call 662 915 1090

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Automatic reply: Hello
Date: Tuesday, September 3, 2013 10:11:52 AM

Dr. Khan is out of office till September 7th if you need assistance please contact Ms. jennifer 662 915 1090 and email jnnfrtyl@olemiss.edu

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Automatic reply: Hello
Date: Thursday, August 13, 2015 2:25:23 PM

Dr. Khan is out of office till August 14th, 2015. Please contact Ms. Jennifer jnnfryl@olemiss.edu or by phone 662 915 1090 if you need immediate assistance.

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Automatic reply: Request for chemical
Date: Monday, July 18, 2016 3:42:56 PM

I am currently out of the office till July 21. Please contact Ms. Jennifer if immediate attention is needed
jnnfrtyl@olemiss.edu

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Automatic reply: thanks from Jing
Date: Tuesday, August 27, 2013 9:37:32 PM

Dr. Khan is out of office till August 30th if you need assistance please contact Ms. jennifer 662 915 1090 and email is jnnfrtyl@olemiss.edu

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Automatic reply: Wishes
Date: Friday, December 25, 2015 12:40:54 PM

Wish Happy Holidays and a Happy New Year. Univeristy will reopen on 4th Jamuray.

From: bavula@olemiss.edu
To: [Pawar, Rahul](#)
Subject: check
Date: Wednesday, May 26, 2010 8:15:13 AM

check the following site....

<http://www.youtube.com/watch?v=mhbgOIHRyQ&feature=channel>

Bharathi

From: Micah Quinn
To: [Pawar, Rahul](#)
Subject: CORRECTED - Greetings from the Oxford ICSB
Date: Wednesday, January 22, 2014 12:32:15 PM

2014_Flyer_Header



Greetings from the ICSB Team,
We would like to remind you all that registration is open for the 13th ICSB . The flyer with information on the Conference's topics is below, and can be downloaded from our website at <http://oxfordicsb.org>.

20th Anniversary of the DSHEA

**The Past, Present and Future of Botanical
Dietary Supplement Quality and Safety**

The National Center for Natural Products Research (NCNPR) within the School of Pharmacy at the University of Mississippi is pleased to announce its 13th Annual Oxford International Conference on the Science of Botanicals.

This conference provides a venue for regulators, academia and industry to discuss current quality and safety issues that impact all stakeholders. This year's symposia will focus on the future of identifying and promoting best practices that enhance the quality and safety of botanical containing products and dietary supplements in commerce. Hear the perspectives of regulators, academics, manufacturers and trade associations. To this end, the program will include relevant presentations from members of CFSAN/FDA, other international regulatory bodies, major trade associations, scientists and industry representatives.

Topics Include:

- **International Regulatory and Corporate Approaches for Safety Assessments**
- **Adulteration: the problem and potential solutions**
- **Post market surveillance/Adverse event reporting and models for causation (association) assessment**
- **Communicating safety to consumers**
- **Application of modern technologies toward establishing the safety, efficacy, and quality of botanicals**

Thanks from the ICSB Web Team!

For more information, please feel free to contact:

Ikhlas Khan, PhD. Director of the FDA Center of Excellence

Assistant Director, NCNPR
School of Pharmacy, University of Mississippi
icsb@olemiss.edu
<http://oxfordicsb.org>
Like us on Facebook!
<http://facebook.com/OxfordICSB>

2014logoblock



If you do not want to receive future emails from Micah Quinn, click [Opt-Out](#).



From: [Pawar, Rahul](#)
To: [Noonan, Gregory](#)
Subject: CV for Postdoc position
Date: Monday, March 14, 2016 11:59:00 AM
Attachments: [Sagi CV-Academics.doc](#)

Hi Greg,

I am forwarding the CV of Satyanarayanaraju Sagi (Sagi) for consideration for the postdoc position in dietary supplements. Thanks,
Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Monday, March 14, 2016 11:49 AM
To: Pawar, Rahul
Subject: RE: Application for research position in your group

Dear Rahul,

Please find the enclosed my updated CV.

Thanks
Sagi

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Monday, March 14, 2016 10:33 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Application for research position in your group

Please send me your updated cv, thanks

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Friday, August 21, 2015 2:50 PM
To: Pawar, Rahul
Subject: RE: Application for research position in your group

Thank you for your reply. It will be really great opportunity for me to work in your team in FDA.

Will keep in touch with you

Thank you
Sagi

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Friday, August 21, 2015 1:43 PM
To: SATYANARAYANARAJU SAGI
Cc: Ikhlas Khan
Subject: RE: Application for research position in your group

Hi Sagi,

Thanks for your interest. We don't have the position available currently but we are working on it. I will keep you posted if this materializes, hopefully by the end of this year.

Best wishes,

Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]

Sent: Friday, August 21, 2015 2:22 PM

To: Pawar, Rahul

Cc: Ikhlas Khan

Subject: Application for research position in your group

Dear Dr. Rahul,

I Satyanarayanaraju Sagi, introduce myself as a Post Doctoral Research Associate, working with Dr. Ikhlas Khan, Associate Director, NCNPR, University of Mississippi, Univeristy, Mississippi, USA. During my post doctoral research program, I have been working in analytical department which mainly focus on development of chemical fingerprint profiles of plant extracts using HPTLC, HPLC, UHPLC and UHPLC-MS/MS, the major applicability of these methods are in analyzing dietary supplements claiming to specific plant extracts.

My doctoral work was carried under the guidance of Dr. R. Nageswara Rao, Chief Scientist in Analytical chemistry division. My doctoral thesis mainly focused on bioanalytical method development and validation of anticancer and antihypersensitive drugs. The methods include novel blood collection technique like dried blood spots and ionic liquid based liquid-liquid microextraction from rat serum. My research work also include method of reverse phase chiral LC-MS for chiral separation and in-vitro disposition of chiral enantiomers. During this period, as an active member in natural product group my contribution involves method development for preparative isolation of bioactive from the plant extracts and development fingerprinting profile, quantification of marker compounds in various medicinal plants. I will be helping the research scientist in structure elucidation of new isolates through fragmentation studies on mass spectrometry and conformation by high resolution mass spectrometric studies.

Having more than eight years of experience in the field of analytical chemistry with various expertise's I am keen in exploring an opportunity to work in the area of natural products and dietary supplements, which unambiguously would provide me an opportunity to further improve my knowledge and skills. Considering the interest to reach new heights in the field of analytical chemistry/dietary supplements and with all confidence that I shall be a potential competitor for Research Position in your research group, herewith I am enclosing my curriculum vitae for your kind perusal.

Hope my experience and skills meet your requirements. I feel privileged to furnish more information if you need.

Thank you for your consideration and I hope to hear from you in the near future.

Sagi

Satyanarayanaraju Sagi
Post Doctoral Research Associate
National Center for Natural Products Research
School of Pharmacy
The University of Mississippi
University, MS 38677
Work: 662 915 7610
Email: ssagi@olemiss.edu; (b) (6)

From: [Pawar, Rahul](#)
To: ikhan@olemiss.edu; bavula@olemiss.edu
Subject: Dietary supplements labeled as blood sugar management (purchased)
Date: Thursday, August 27, 2015 11:28:00 AM
Attachments: [Dietary supplements labeled as blood sugar management \(purchased\).docx](#)

Hello!

Here is the list of the sugar management products we purchased. These were purely chosen based on the label claim and not by ingredient. So, for example, we have not collected supplements just selling Moringa extract, unless it made a health related claim.

Let me know if you can analyze these products for their botanical ingredient? Thanks.

Best wishes,

Rahul

From: [Pawar, Rahul](#)
To: ikhan@olemiss.edu
Subject: DMAA paper
Date: Friday, February 14, 2014 3:08:00 PM

FYI

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3682735/pdf/aci-8-2013-029.pdf>

Happy Valentine's day!

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: Tamta, Hemlata *
To: [Pawar, Rahul](#)
Subject: Dr. Herath's message
Date: Wednesday, October 15, 2014 8:39:56 AM

Bubs,

Dr. Herath's wife had sent the following message. Please contact him.

Hi Hemlata, how are you all doing? My husband wants to contact Rahul . I think he has left a message. please check it and could you please let him send an email to bherath@olemiss.edu

Hemlata Tamta, Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1959
Fax: 301-436-2622

From: Jennifer S. Taylor
To: [Pawar, Rahul](#)
Cc: [Ikhlas Khan](#)
Subject: Dr. Khan's trip to MD
Date: Thursday, August 30, 2012 9:47:47 AM

Rahul,

Dr. Khan asked me to send you his itinerary and hotel information. He said to tell you that if you were unable to meet him when he arrives he would like for you to meet him the next day in the morning. This is his cell phone number: (b) (6).

Delta flight **#DL1938** from Atlanta to Washington arriving at **6:06pm**.

He will be staying at **Greenbelt Marriott**, 6400 Ivy Lane, Greenbelt, MD 20770.

Please let me know if you have any questions.

Sincerely,

Jennifer Taylor

Jennifer Taylor
Senior Secretary
University of Mississippi
National Center for Natural Products Research
301Q Thad Cochran
P.O. Box 1848
University, MS 38677

✉ jnnfrtyl@olemiss.edu
☎ 662-915-1090
📠 662-915-7989

From: [Pawar, Rahul](#)
To: [Tamta, Hemlata *](#)
Subject: Dr. Walker Reco for Pawar
Date: Wednesday, April 4, 2012 1:15:00 PM

April 04, 2012
Peer-review committee
CFSAN, FDA

RE: Recommendation for Dr. Rahul Pawar

Dear Peer-review committee,

This letter is in support of Dr. Rahul Pawar's application for promotion to GS-14 grade. Dr. Pawar was a Postdoctoral Research Associate in our center from June 2003 to November 2007, and worked in our FDA-sponsored program on the isolation and identification of the active principles of medicinal plants and botanical supplements including many dietary supplements. Though still relatively early in his career, Dr. Pawar's resume will show that he is a very accomplished researcher with a solid academic background and substantial research experience.

During his early experience with us, he brought an exceptional set of natural product isolation and structure elucidation skills and a great deal of maturity and independence. In our Center's cooperative agreement with the U.S. Food and Drug Administration, one of the important responsibilities is to understand the chemical constitution of plants that might carry health risks. Several of these products find their way into the U.S. market, usually claiming to help with weight loss, sexual performance and metabolic disorders. Dr. Pawar led the isolation chemistry efforts on several of these, and as evidenced in his curriculum vitae, has been extremely productive in his 4 years with us. He quickly assumed a leadership role in our natural products isolation, taking responsibility for new instrumentation, training of students and new postdoctoral fellows, and solving difficult problems in the structure elucidation of natural products.

Dr. Pawar was involved in several of our research programs. Dr. Pawar's field of study was especially important for our understanding of medicinal plants. Many of these constituents find their way, either intentionally or as adulterants, into the U.S. market place and understanding their chemical composition is critical. He played a key role in the investigations of traditional medicinal plants that were suspected to contain the hepatotoxic aristolochic acid. He also played a lead role in chemical investigation of *Hoodia gordonii* plant. His work has lead way for more than fifteen high valued publications on *Hoodia*. Our efforts on Hoodia have been praised by regulatory agencies, trade organizations and industries across the world and I am glad to recognize Dr. Pawar's contribution.

Dr. Pawar brought an exceptional element in the quality of his character, work ethic, and collegiality. He interfaced well with other scientists and co-workers at all levels, and thus exhibited solid leadership for our growing program. He worked very long hours, and was committed to his job and the program and people he worked with. He very positively impacted the work environment and the work productivity of others around him.

Dr. Pawar was identified as a lead exchange scientist for our FDA cooperative agreement. Since the end of 2007, Dr. Pawar has been working with CFSAN-FDA's Washington DC office to facilitate the interaction between our laboratories and mutual program interests. I am sure his strong background and research skill is valued asset to the agency.

I recommend Dr. Pawar's promotion. I would be happy to answer any questions you may have.

Sincerely,

Larry A. Walker
Director, NCNPR
University of Mississippi
University, MS 38677
E-mail: ncnpr@olemiss.edu
Phone 662-915-1005

From: [Pawar, Rahul](#)
To: ikhan@olemiss.edu
Subject: Duke database
Date: Monday, October 26, 2015 11:26:00 AM

Hello Dr. Khan,

Just came to know something about James Duke's NP database which I thought you might be interested in. USDA will not be hosting and updating the database by this yearend and Dr. Duke is offering it to anyone who might be interested in hosting it. Let me know if UM is interested in having it, I will provide more information as I get. TTY soon.

Best wishes,

Rahul

Rahul Pawar, Ph.D.

Office of Regulatory Science

CFSAN-FDA

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 240-402-1795

Fax: 301-436-3077

From: [Pawar, Rahul](#)
To: [Ma, Jun](#)
Subject: FW: <no subject>
Date: Monday, September 10, 2012 3:35:43 PM
Attachments: [J Guizhou Inst Tech 1996 1.pdf](#)

Hi Jun,
Attached is the paper I need translation on, when you have time please help with this.
Rahul

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Tuesday, August 21, 2012 11:07 PM
To: Pawar, Rahul
Subject: <no subject>

Thanks, I will be coming to attend ceremony and congratulations to you too. I should be arriving on 6th evening and will leave on 7th in the evening.
IK

From: [Pawar, Rahul *](#)
To: [Tamta, Hemlata *](#)
Subject: FW: Botanicals
Date: Tuesday, September 6, 2011 12:24:00 PM

From: VIJAYASANKAR RAMAN [mailto:vraman@olemiss.edu]
Sent: Friday, September 02, 2011 2:42 PM
To: Pawar, Rahul *
Subject: RE: Botanicals

Dear Rahul

I have not received any information from Dr. Khan regarding sending of samples to you. And he is currently out of station.

Vijay.

From: Pawar, Rahul * [Rahul.Pawar@fda.hhs.gov]
Sent: Friday, September 02, 2011 1:36 PM
To: VIJAYASANKAR RAMAN
Subject: RE: Botanicals

Hi Vijay,
Please let me know when you ship the material.
Thanks have a good weekend
Rahul

From: VIJAYASANKAR RAMAN [mailto:vraman@olemiss.edu]
Sent: Tuesday, August 30, 2011 3:31 PM
To: Ikhlas Khan; Aruna Weerasooriya
Cc: Pawar, Rahul *
Subject: RE: Botanicals

Dear Rahul

We currently have authenticated samples of three species - *Acorus calamus*, *Piper methysticum* and *Tripterygium wilfordii*; and two commercial samples (not authenticated) viz. *Larrea tridentata* and *Teucrium chamaedrys*. If needed, I can help you with authentication of these samples.

Regards

Vijay

From: Ikhlas Khan
Sent: Tuesday, August 30, 2011 1:23 PM
To: Aruna Weerasooriya; VIJAYASANKAR RAMAN
Cc: Rahul
Subject: FW: Botanicals

Thanks Rahul

Aruna and Vijay will give you update on this. They have most of it but I am not sure which one.

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>

Date: Tue, 30 Aug 2011 13:05:59 -0400

To: Ikhlas Khan <ikhlan@olemiss.edu>

Subject: RE: Botanicals

Hello Dr. Khan,
Happy ID to you all.
Any update about the plant materials?
Thank you
Rahul

From: Ikhlas Khan[<mailto:ikhlan@olemiss.edu>]

Sent: Tuesday, August 09, 2011 3:04 PM

To: Pawar, Rahul

Subject: Re: Botanicals

It was our pleasure. We all enjoyed to be with your family. I will check with Vijay and Aruna and come back to you. I am sure we have most of them.

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>

Date: Tue, 9 Aug 2011 10:56:21 -0400

To: Ikhlas Khan <ikhlan@olemiss.edu>

Subject: Botanicals

Hello Dr. Khan,
I hope you all are doing well. It was great to spend some wonderful time with you all at San Diego. I was in need of the following authenticated botanical materials (about 100 gm each), can you please provide them to us? If you are unable to provide some of these then I can buy this online (like Frontier Coop or Starwest) but then can any botanist on your lab help in the authentication?

1. Germander: *Teucrium chamaedrys*
2. Calamus root: *Acorus calamus*
3. Kava Kava: *Piper methysticum*
4. Chaparral: *Larrea tridentata*
5. Usnea: *Usnea* sp
6. Cascara sagrada bark
7. *Tripterygium wilfordii*

Please let me know if that is possible. Thank you.

Best regards,

Rahul

Rahul Pawar Ph.D.

Division of Bioanalytical Chemistry

CFSAN-FDA

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 240-402-1795

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: FW: ICSB coverage from Nutraingredients-usa.com
Date: Friday, April 15, 2016 5:32:53 PM

Told you so
ik

From: "frank@unpa.com" <frank@unpa.com>
Date: Friday, April 15, 2016 at 10:35 AM
To: Loren Israelsen <loren@unpa.com>
Cc: Ikhlas Khan <ikhlan@olemiss.edu>, Peter Reinecke <peter@rssinc.us>, Patricia Knight <pknight@knight-cap.us>
Subject: ICSB coverage from Nutraingredients-usa.com

Data confirms that DNA barcoding alone not suitable for finished dietary supplement products:
http://www.nutraingredients-usa.com/Research/Data-confirms-that-DNA-barcoding-alone-not-suitable-for-finished-dietary-supplement-products/?utm_source=newsletter_daily&utm_medium=email&utm_campaign=15-Apr-2016&c=6htVoOGdDM2Ft9cj6P41qV9epLetwIXu&p2=

FDA ‘appreciates’ CRN’s product registry efforts/Sharfstein presentation:
http://www.nutraingredients-usa.com/Regulation/FDA-appreciates-CRN-s-product-registry-efforts/?utm_source=newsletter_daily&utm_medium=email&utm_campaign=15-Apr-2016&c=6htVoOGdDM0ER4G00d4eyX%2Fy72wRbm9o&p2=

From: Jennifer S. Taylor
To: [Pawar, Rahul](#)
Cc: [Ikhlas Khan](#)
Subject: FW: Last Minute Information about Your Trip
Date: Thursday, September 6, 2012 3:40:34 PM

Fyi

From: Delta Messenger [mailto:DeltaMessenger@delta.com]
Sent: Thursday, September 06, 2012 2:34 PM
To: Ikhlas Khan
Subject: Last Minute Information about Your Trip

A time change has occurred for your Delta flight. Please see flight info.

[Delta.com](#) | [My Itinerary](#) | [Add to Address Book](#)
Ikhlas Khan SkyMiles (b) (6)

The times for your flight(s) have changed. Please review your updated flight information.

Delta Confirmation #GAJMC8

Dear Ikhlas Khan,

We are trying to contact you because the times have changed for your flight on September 6. We'll look in your reservation for a telephone number and try to reach you there too. Please review the information below for new flight numbers, departure and arrival times.

Note: If your departure is from an International city, please check in for the original departure time

[Join SkyMiles](#) | [Flight Status](#) | [View Itinerary](#)

UPDATE		
Thursday, September 6 Flight Delta 1938	Departs 5:00 pm Atlanta, Georgia 4:10 pm Arrives 6:47 pm Washington - Reagan National, Washington DC 6:06 pm	Seat 04B

Please see airport monitors for gate information.

We apologize for this interruption in your travel plans. You can check-in online or at one of our self service kiosks. If you already have your boarding pass, just scan the bar code at one of our boarding scanners or kiosks to receive your updated travel document.

If you have any questions, please contact [Delta Reservations](#), or go online to check your [flight status](#).

Thank you for choosing Delta.

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Delta Blvd. P.O. Box 20706 Atlanta, GA 30320-6001

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Cc: [Ikhlas Khan](#); [ZULFIQAR ALI](#)
Subject: FW: Mitragyna javanica
Date: Thursday, October 16, 2014 10:18:24 AM

We do not have M. javanica and Vijay tried but could not get

**thanks
bharathi**

From: VIJAYASANKAR RAMAN
Sent: Thursday, October 16, 2014 8:58 AM
To: BHARATHI AVULA
Subject: RE: Mitragyna javanica

Bharathi,

We don't have M. javanica.

Vijayasankar Raman, Ph.D.
Systematic Botanist
National Center for Natural Products Research
Room # 3027, Thad Cochran Research Center
School of Pharmacy
University of Mississippi
University, MS-38677
Ph. (662) 915-1018

From: BHARATHI AVULA
Sent: Thursday, October 16, 2014 7:57 AM
To: VIJAYASANKAR RAMAN
Cc: Shobhan Singh (ssingh2@go.olemiss.edu)
Subject: Mitragyna javanica

Dear Vijay,

Could you please check and let me know if we have samples of Mitragyna javanica

**Thanks
bharathi**

From: Ikhlas Khan
Sent: Thursday, October 16, 2014 7:54 AM
To: BHARATHI AVULA; Pawar, Rahul (Rahul.Pawar@fda.hhs.gov)
Cc: ZULFIQAR ALI
Subject: Re: Kratom

We need to get the sample to confirm it.
ik

From: Bharathi Avula <bavula@olemiss.edu>
Date: Thursday, October 16, 2014 at 7:01 AM
To: Rahul Pawar <Rahul.Pawar@fda.hhs.gov>

Cc: Ikhlas Khan <ikhlan@olemiss.edu>, ZULFIQAR ALI <zulfiqar@olemiss.edu>

Subject: FW: Kratom

Dear Rahul,

From literature search, the *M. javanica* did not contain mitragynine (C₂₃H₃₀N₂O₄; 398.2205) but contains javaphylline (C₂₂H₂₆N₂O₅; 398.1841), mitrajavine...

Thank you
bharathi

From: Ikhlas Khan
Sent: Thursday, October 16, 2014 3:55 AM
To: BHARATHI AVULA; ZULFIQAR ALI
Subject: Fwd: Kratom

Can you respond to rahul

Sent from my iPhone

Begin forwarded message:

From: "Pawar, Rahul" <Rahul.Pawar@fda.hhs.gov>
Date: October 16, 2014 at 4:30:52 AM GMT+8
To: "ikhlan@olemiss.edu" <ikhlan@olemiss.edu>
Subject: Kratom

Hello Sir,
One quick question. Does Mitragyna javanica contain Mitragynine?
Thanks
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: [Tamta, Hemlata](#) *
Subject: FW: News about Valli
Date: Thursday, August 23, 2012 9:20:56 AM

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Thursday, August 23, 2012 8:18 AM
To: Pawar, Rahul; (b) (6) @gmail.com
Subject: RE: News about Valli

I am sorry to write that Valli passed away....

Bharathi

From: NCNPR
Sent: Wednesday, August 22, 2012 3:17 PM
Subject: News about Valli

To: NCNPR Faculty & Staff, USDA and School of Pharmacy

Dear NCNPR faculty and staff,

This afternoon, we learned that Rangavalli "Valli" Manyam has passed away. When she (b) (6)

[REDACTED]
[REDACTED]. Though not entirely unexpected, I know all of us are saddened, and we remember her many contributions to our family here. So many of you have helped her unselfishly in the last couple of years, and I want to thank all of you for your compassion and care for her.

As the final arrangements are available, we will try to forward that information.

Larry Walker, Director

National Center for Natural Products Research
Thad Cochran Research Center, Room 1019
University of Mississippi
P. O. Box 1848
University, MS 38677
Phone: 662-915-1005
Fax: 662-915-1006

From: [Brown, Detra](#)
To: [Pawar, Rahul](#)
Cc: [Noonan, Gregory](#)
Subject: FW: ORISE - Satyanarayanaraju Sagi
Date: Monday, July 25, 2016 7:53:48 AM
Attachments: [ORISE Application -Sagi.doc](#)
[Employment letter-Dr. Khan1.pdf](#)
[Sagi CV-Academics.doc](#)
[Sagi-Educational Certificates.pdf](#)
[Sagi-Reference Let- FDA-Orise appl.pdf](#)
[Sagi-Insurance details.pdf](#)
[2016 ORISE New Supplements.doc](#)
[SKMBT_C36016072507550.pdf](#)

Good Morning,

FYI – package has been sent to Tashea Brown for further processing.

Thanks

Detra

From: Brown, Detra
Sent: Monday, July 25, 2016 7:53 AM
To: Brown, Tashea D (Tashea.Brown@fda.hhs.gov)
Subject: ORISE - Satyanarayanaraju Sagi

Tashea,

Attached, please find the necessary documents for processing. Originally submitted as TBD for FY16, attached in WORD and PDF to capture the signature page. If I can assist further, please let me know.

Thanks

*Detra Brown
Management Analyst
Food and Drug Administration
Center for Food Safety
and Applied Nutrition
Office of Regulatory Science
5001 Campus Drive
College Park, MD 20740
240-402-3004 (P)
301-436-2332 (F)*

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: FW: Photo from CFSAN Awards
Date: Saturday, September 8, 2012 8:55:02 PM
Importance: High

Hi Rahul

We arrived safely. I received this photo I know barbara and Mike but don't know other two people in it. Can you give their name and designation.

Thanks

IK

From: bill mindak <(b) (6) @yahoo.com>
Reply-To: bill mindak <(b) (6) @yahoo.com>
Date: Sat, 8 Sep 2012 08:55:51 -0700
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Photo from CFSAN Awards

Hi Iklas,

Nice to meet you yesterday. Attached is your photo from the CFSAN awards ceremony.

Bill Mindak

From: [Pawar, Rahul](#)
To: (b) (6) [@yahoo.com](#)
Subject: FW: Rec letter for Pawar
Date: Monday, June 6, 2011 4:21:00 PM
Attachments: [June 6 2011 letter for Rahul Pawar.pdf](#)

From: Larry Walker [mailto:lwalker@olemiss.edu]
Sent: Monday, June 06, 2011 4:16 PM
To: Pawar, Rahul
Subject: FW: Rec letter for Pawar

Rahul,
Here is the recommendation letter. Hope it helps!
Best regards,
Larry Walker

From: [Pawar, Rahul](#)
To: [Tamta, Hemlata](#) *
Subject: FW: some more pics
Date: Friday, August 12, 2011 3:16:00 PM
Attachments: [DSC00106.JPG](#)
[DSC00104.JPG](#)
[DSC00105.JPG](#)
[DSC00110.JPG](#)
[DSC00109.JPG](#)
[DSC00114.JPG](#)
[DSC00113.JPG](#)

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Friday, August 12, 2011 1:15 PM
To: Pawar, Rahul
Subject: some more pics

Please find enclosed few more pics

bharathi

From: [Pawar, Rahul](#)
To: [Tamta, Hemlata *](#)
Subject: FW: Thank you
Date: Monday, August 2, 2010 10:56:00 AM

From: Larry Walker [mailto:lwalker@olemiss.edu]
Sent: Monday, August 02, 2010 9:46 AM
To: Pawar, Rahul
Subject: RE: Thank you

Rahul

Congratulations! I am so happy for you. You and your family are a great credit to us and to this country, as well as your own. God bless you all.

Larry

Larry A. Walker, Ph.D.
Director
National Center for Natural Products Research
School of Pharmacy
University of Mississippi
University, MS 38677
USA
Phone: 662-915-1005
Fax: 662-915-1006

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From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Monday, August 02, 2010 8:41 AM
To: 'lwalker@olemiss.edu'
Subject: Thank you

Hi Dr. Walker,

I hope you are doing well.

I am happy to inform you that my petition for the permanent residency was accepted. I am sure your recommendation had a very positive impact on my application and I want to express my gratitude for your kind and timely help with the letters.

Please do not hesitate to ask if I can be any help to you.

Best regards

Rahul

From: [Pawar, Rahul](#)
To: ikhan@olemiss.edu; [Rader, Jeanne I *](#)
Subject: FYI - DMAA
Date: Friday, December 19, 2014 8:54:00 AM

Abuse liability of the dietary supplement dimethylamylamine

<http://www.sciencedirect.com/science/article/pii/S0376871614019188>

Rahul Pawar Ph.D.
Office of Regulatory Science
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-3077

From: [Pawar, Rahul](#)
To: ikhan@olemiss.edu
Subject: FYI
Date: Friday, May 8, 2015 9:05:00 AM

http://www.washingtonpost.com/news/morning-mix/wp/2015/05/08/the-brutal-harvest-of-indias-suicide-tree/?tid=hp_mm&hpid=z3

Rahul Pawar, Ph.D.

Office of Regulatory Science

CFSAN-FDA

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 240-402-1795

Fax: 301-436-3077

From: bavula@olemiss.edu
To: [Pawar, Rahul](#)
Subject: hai
Date: Monday, March 28, 2011 2:19:28 PM

hai dear Rahul,

how are you???

I hope this time also all of you are planning to visit oxford for FDA conference....

Best wishes
Bhaarthi

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: hai
Date: Friday, February 21, 2014 10:50:30 AM

Dear Rahul and Hema,

I am back to work. (b) (6) is slowly recovering

**Thank you
bharathi**

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: Happy New Year
Date: Friday, January 18, 2013 10:01:27 AM

Happy new year to you all...

I hope everything is going well from your side...

Best wishes

Bharathi

NOTE: Mail me the following paper:

[Quantitative determination of alkaloids from roots of Hydrastis canadensis L. and dietary supplements using ultra-performance liquid chromatography with UV detection:](#)

Avula, Bharathi; Wang, Yan-Hong; Khan, Ikhlas A. Journal of AOAC International (2012), 95(5), 1398-1405

From: [Pawar, Rahul](#)
To: ikh@olemiss.edu
Subject: Hello
Date: Wednesday, February 11, 2015 4:24:00 PM

Hello Dr. Khan.

I tried to call you couple of times and then came to know from Bharathi that you are travelling to India. Congratulations for the award!

I spoke with my manager and there is positive response for starting a antidiabetic botanical supplement project with NCNPR. Hope to talk with you more when you are back.

Best regards

Rahul

Rahul Pawar Ph.D.
Office of Regulatory Science
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-3077

From: [Pawar, Rahul](#)
To: bavula@olemiss.edu
Subject: Hello
Date: Tuesday, February 4, 2014 10:46:00 AM

Hi Bharathi,
Hope you are back, How was your vacations?
Will talk to you soon.
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: ikhana@olemiss.edu
Subject: Hello
Date: Thursday, September 19, 2013 8:30:43 AM

Hello Sir,
I heard you are in DC.
Just want to update that Jing li will be joining us in January 14.
Thanks
Rahul

From: [Pawar, Rahul](#)
To: ["mstrawn@olemiss.edu"](mailto:mstrawn@olemiss.edu)
Subject: Hi
Date: Wednesday, April 28, 2010 9:11:57 AM

Dear Kathy,

I hope you are doing well.

It was wonderful to see you at oxford, I am so glad we all could make it to oxford.

By the way when are you retiring?

Can you please do me a favor, I want a recent CV of Dr Khan for drafting a recommendation letter.

Please send one when you have time.

Sorry, I will send the pictures to you soon.

Also please send me your personal e-mail/phone number

Best Regards

Rahul

Rahul Pawar Ph.D.

Division of Bioanalytical Chemistry

CFSAN-FDA

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 301-436-1795

Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: ["tsmillie@olemiss.edu"](mailto:tsmillie@olemiss.edu)
Subject: Hi
Date: Monday, May 2, 2011 4:51:00 PM

Hi Troy,

It was very sad to hear the news about the damage to your house in the recent tornado (b) (6). I was relieved to know that you and your family were unharmed but it must be an immense loss and stress to all of you. Please let us know if we can be of any assistance during these tough times.

Best regards

Rahu (b) (6)

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 301-436-1795
Fax: 301-436-2622

From: BHARATHI AVULA
To: [HEMANT LATA](#); [SUMAN CHANDRA](#); [PREMALATHA BALACHANDRAN](#); [Vaishali Joshi \(Vaishali.Joshi@iovate.com\)](#); [Pawar, Rahul](#); [RANGAVALLI B MANYAM](#); [ZULFIOAR ALI](#); [Waseem Gul \(wgul@elsohly.com\)](#); [SRIDEVI ANKISSETTY](#); [SUMITHRA ROHINIE WEERASOORIYA](#); [Aruna Weerasooriya](#)
Subject: Home
Date: Monday, February 27, 2012 8:04:23 AM

Dear All,

During the weekend I have moved into (b) (6) . I thank you all for kind support and wishes.

Bharathi

From: ICSB Web Team
To: [Pawar, Rahul](#)
Subject: Hotel Booking and Final Agenda!
Date: Tuesday, April 5, 2016 10:04:30 AM

ICSB Header Generic



Joint Meeting with 16th Annual International Conference on the Science of Botanicals (ICSB) & 5th Interim American Society of Pharmacognosy (ASP)

Greetings from the ICSB Web Team!

Firstly, be aware that hotel rooms are booking rapidly! The hotels located next to the conference center are both full, but there are still some rooms available at the [Hampton Inn West Oxford](#) and the [Holiday Inn Express](#). Be sure to mention the **Botanical Conference** when you book, or you may be told there are no rooms available as they are holding blocks just for us. These are the only hotels that will have official shuttle service to and from the conference center, so book fast!

We would like to announce that the final agenda for the 16th ICSB and 5th interim ASP meeting is available for viewing and download now at <http://oxfordicsb.org/index.php/agenda>.

Poster guidelines are also available at <http://oxfordicsb.org/index.php/submissions> so be sure to take a look at those if you have submitted a poster this year.

You can still register [via our CVent page](#). As always, feel free to direct any questions you may have to us at ICSB@olemiss.edu, and don't forget to visit our site for further info, and follow us Facebook for up-to-the-minute announcements and updates (Facebook.com/OxfordICSB).

Thank you,

ICSB Web Team,
<http://oxfordicsb.org>
<http://facebook.com/OxfordICSB>
icsb@olemiss.edu

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If you no longer want to receive emails from Micah Quinn please click the link below.

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From: Jennifer S. Taylor
To: [Pawar, Rahul](#)
Subject: ICSB speaker request
Date: Monday, March 14, 2016 5:36:09 PM

Dr. Pawar,

Can you please send me a one page biosketch for the booklet. I would like to finish the booklet this week.

Thanks,

Jennifer Taylor

Jennifer Taylor
Program Coordinator
University of Mississippi
National Center for Natural Products Research
3012 Thad Cochran
P.O. Box 1848
University, MS 38677
 jnnfrtyl@olemiss.edu
 662-915-1090
 662-915-7989

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From: ICSB Web Team
To: [Pawar, Rahul](#)
Subject: ICSB Special Session Announcement
Date: Wednesday, March 25, 2015 5:37:43 PM

ICSB Header Generic



15th Annual Oxford International Conference on the Science of Botanicals

Greetings from the ICSB Web Team!

On February 3rd, 2015 the Attorney General of New York issued cease and desist letters to 4 major US retailers alleging that botanical dietary supplements sold under their house brands contained virtually none of the ingredients listed on the label. The test method used was DNA barcoding. This triggered an ongoing national media story, class action lawsuits, other state AG's following the lead of New York, and a great deal of criticism over the use of DNA barcoding to test botanical extracts.

The implications of this are far reaching, both in terms of new scrutiny of the global botanical supply chain, contract manufacturer competence, proper use of analytical testing methods, legal and political consequences and the reputation of the dietary supplement industry.

In light of these developments, a special 90 minute session will be held at the **15th International Conference on the Science of Botanicals (ICSB)**, being held April 13th-16th, 2015 in Oxford, MS, taking advantage of the gathering of scientists, regulators and industry representatives. The purpose will be to detail what has happened, what is being done by industry, retesting programs under way, and much more.

This session will be moderated by a panel of industry experts, including:

- **Loren Israelsen** - President - United Natural Products Alliance
- **Mark Blumenthal** - Executive Director - American Botanical Council
- **Nandajumara Sarma** - Director / Dietary Supplements - US Pharmacopeia

For more information, visit our site at <http://oxfordicsb.org>. Also, don't forget to register as soon as possible! Rates go up sharply for on-site registration, so be sure to book online. You can register [via our CVent page](#). As always, feel free to direct any questions you may have to us at ICSB@olemiss.edu, and don't forget to visit our site for further info, and follow us Facebook for up-to-the-minute announcements and updates ([Facebook.com/OxfordICSB](https://www.facebook.com/OxfordICSB)).

Thank you,

ICSB Web Team,
<http://oxfordicsb.org>
<http://facebook.com/OxfordICSB>
icsb@olemiss.edu

emailblock

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If you no longer want to receive emails from Micah Quinn please click the link below.

[Opt-Out](#)



From: [Pawar, Rahul](#)
To: amar@olemiss.edu
Subject: ICSB
Date: Friday, December 2, 2016 2:49:00 PM

Hi Amar,
Hope all is well. Are there any plans for extending the deadline for abstract submission? Thanks,
Rahul

Rahul Pawar, Ph.D.
Research Chemist
Office of Regulatory Science/DBC/BMB
FDA/Center for Food Safety and Applied Nutrition
5001 Campus Drive
College Park, MD 20740
Tel: 240-402-1795

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: Images of products
Date: Thursday, December 11, 2014 8:01:35 AM

Find enclosed the pictures of products.

Thanks
bharathi

From: Jennifer S. Taylor
To: [Atish Paul](#); [Atul Jadhav](#); [chidananda swamy Rumalla](#); [Dr. Vijai K Agnihotri](#); [earla ravinder](#); [Ehab](#); [Erdal Bedir](#); [Feng Wei](#); [Hyung-in Moon \(himun68@dau.ac.kr\)](#); [Jamal Mustafa](#); [jamal mustafa mustafa](#); [Julius Ngunde Ngwendson](#); [li jing](#); [MATSUZAKI Keiichi \(matsuzaki.keiichi@nihon-u.ac.jp\)](#); [Nurdan S Duzgoren-Aydin \(naydin@njcu.edu\)](#); [Pawar, Rahul](#); [Sara Crockett](#); [Sridhar Rao Ayinampudi](#); [Toshiaki Makino \(makino@phar.nagoya-cu.ac.jp\)](#); [Vaishali Joshi](#); [vamsi.m](#); [wangwei](#); [yatin shukla](#); [Young-Whan Choi \(ywchoi@pusan.ac.kr\)](#)
Cc: [Ikhlas Khan](#)
Subject: information request
Date: Thursday, September 6, 2012 12:01:44 PM

Dr. Khan would like to have your current contact information along with your current employment information. He is working on a project and needs to have this info. Also, if you happen to know any contact info for other that do not appear on my list, please provide it for me so that I can have a complete list of past group members. Thank you for your time and cooperation.

Jennifer Taylor

Jennifer Taylor
Senior Secretary
University of Mississippi
National Center for Natural Products Research
301Q Thad Cochran
P.O. Box 1848
University, MS 38677

✉ jnnfrtyl@olemiss.edu
☎ 662-915-1090
☎ 662-915-7989

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: JMS
Date: Friday, April 5, 2013 8:07:29 AM

Do you by any chance have access to Journal of Mass Spectrometry???

Are you coming for our upcoming conference??

bharathi

From: [Pawar, Rahul](#)
To: ["lwalker@olemiss.edu"](mailto:lwalker@olemiss.edu)
Subject: letter Request
Date: Wednesday, April 4, 2012 2:57:00 PM
Attachments: [Dr. Walker Reco for Pawar.doc](#)

Dear Dr. Walker,

I hope you are doing fine.

I need a favor from you. I have to submit application for a peer-review at FDA for promotion to grade GS-14. Can you please provide me a recommendation letter for the same?

A draft is attached, please feel free to edit it.

As always thank you for the help.

Best regards

Rahul

Rahul Pawar Ph.D.

Division of Bioanalytical Chemistry

CFSAN-FDA

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 240-402-1795

Fax: 301-436-2622

From: bavula@olemiss.edu
To: [Pawar, Rahul](#)
Subject: letters
Date: Friday, September 17, 2010 8:53:21 AM

I did not get any mail from you about the letters (mail me your covering letter also...)

Did you apply for EB1-A or B ?????

Thank you
Bharathi

From: [Pawar, Rahul](#)
To: [SATYANARAYANARAJU SAGI \(ssagi@olemiss.edu\)](mailto:ssagi@olemiss.edu)
Date: Wednesday, May 25, 2016 8:29:00 AM
Attachments: [Interview Travel Form_FF.PDF](#)

Raju,

Can you please fill out this form, as much as you can, and send it back to me.

Roughly, you can reserve time from 9 to 3 pm on 22nd June for the presentation, interview, lunch and meeting here.

You can choose travel to any Washington airports (IAD, DCA, BWI) and then take a shuttle, taxi or metro to reach a hotel in College Park or Beltsville MD area.

Let me know if you need more information.

Thanks,

Rahul

From: [Pawar, Rahul](#)
To: ["bavula@olemiss.edu"](mailto:bavula@olemiss.edu)
Date: Wednesday, March 13, 2013 9:40:53 AM
Attachments: [s2.pdf](#)

Here you go..

From: Aruna Weerasooriya
To: [Pawar, Rahul](#)
Date: Friday, September 14, 2012 1:19:47 PM

Dear Rahul,

Sorry for the delay in getting back to you. Today only I saw your voice mail because the telephone and internet system in the new building had some issues and all of a sudden I started getting all voice mails from the server. Anyway, We do not have any plans or pictures of Blue Cohosh. I actually have nothing to do with that DC conference. The organizers have put my name there without even asking but I have no time for these things! Things are very hectic here without enough staff and I am very near to give this up!!!

Best,

Aruna

From: [Pawar, Rahul](#)
To: [Shah, Romina](#)
Date: Tuesday, April 10, 2012 2:01:00 PM

<http://pharmacy.olemiss.edu/php/sopquery3.php?id=226>

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: Ikhlas A. Khan
To: [Pawar, Rahul](#)
Date: Thursday, August 19, 2010 12:35:50 PM

Hi Rahul

Just to let you know, I am flying on 26th and should be arriving at Washington National at 5:48 P.M.

My hotel has been booked, Greenbelt Marriott 6400 Ivy Lane, Greenbelt, Maryland 20770 USA Phone: 1-301-441-3700. I will be flying back on Saturday morning at 11:35 a.m.

From: [Pawar, Rahul](#)
To: ["Premalatha Balachandran \(prembala@olemiss.edu\)"](#)
Date: Thursday, May 20, 2010 11:40:09 AM

Hi Premalatha,
How are you doing?
Do you have pdf for the following paper?

Pawar, R. S., Balachandran, P., Pasco, D. S., and Khan, I. A. 2006. Cytotoxicity studies of triterpenoids from *Akebia trifoliata* and *Clematis ligusticifolia*. *Acta Horticulturae*, 720: 171-178.

Looks like the membership for samsclub is due sometime next month. Do you want me to pay this time?

Thank you
Rahul

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: manuscript title
Date: Friday, April 5, 2013 8:13:24 AM

Please see below:

Profiling primaquine metabolites in primary human hepatocytes using UHPLC-QTOF-MS with ^{13}C stable isotope labeling. Avula, Bharathi; Tekwani, Babu L.; Chaurasiya, Narayan D.; Nanayakkara, Np dhammika; Wang, Yan-Hong; Khan, Shabana I.; Adelli, Vijender R.; Sahu, Rajnish; Elsohly, Mahmoud A.; McChesney, James D.; et al. Journal of Mass Spectrometry (2013), 48(2), 276-285.

Even I am not planning to go for ASP meeting. This time also Vaishali is coming to ICSB conference. Markus also will be here.

Thank you for your help

Bharathi

From: Ikhlas Khan
To: [Fabricant, Daniel](#); [Calvey, Elizabeth M](#); [Rader, Jeanne L](#); [Dou, Jinhui](#)
Subject: news
Date: Monday, March 5, 2012 10:18:10 AM

Hi

I would to share the news with you. I will be awarded Honorary degree (Honoris Causa) D.Sc from University of Hamdard, Delhi, India this weekend. I would like to thank for your support.

Thanks

IK

From: [Pawar, Rahul](#)
To: ikhan@olemiss.edu
Subject: news
Date: Friday, December 4, 2015 8:51:00 PM

FYI

<https://www.pharmamedtechbi.com/publications/the-tan-sheet/23/49/fda-dietary-supplement-division-soon-will-need-office-accommodations?elsca1=tan&elsca2=newsltr>

From: BHARATHI AVULA
To: [Rahul Pawar \(rahulpawar4@yahoo.com\)](mailto:Rahul.Pawar_rahulpawar4@yahoo.com)
Cc: [Pawar, Rahul](#)
Subject: OTC ingredient document
Date: Thursday, June 13, 2013 9:18:10 AM

Is it possible to get the following document:

http://www.fda.gov/cder/Offices/OTC/Ingredient_List_A-C_July_2003.pdf

thank you for your help

bharathi

From: Steven Hopper
To: aali@olemiss.edu; aeichner@usada.org; amar@olemiss.edu; amgalalv@olemiss.edu; anabant@olemiss.edu; andrew.nerlinger@gmail.com; arahman@olemiss.edu; arunaw@olemiss.edu; bavula@olemiss.edu; bherath@olemiss.edu; bhushan.patwardhan@friht.org; bridget.molloy1@gmail.com; [Briggs, Josephine P \(NIH\)](mailto:Briggs, Josephine P (NIH)); btekwani@olemiss.edu; chi@mail.shcnc.ac.cn; chjfp@olemiss.edu; cyalaman@olemiss.edu; daguo@mail.shcnc.ac.cn; dfchen@shmu.edu.cn; dfs@olemiss.edu; dhammika@olemiss.edu; dirnbg@sltnet.lk; dliyan@olemiss.edu; dmackay@crnusa.org; dogglesby@olemiss.edu; dpasco@olemiss.edu; DROBERTS@co.napa.ca.us; eike.reich@camag.com; elsohly@elsohly.com; emcroom@olemiss.edu; ezrab@herbalife.com; fabien.scorza@camag.com; fengqiu2000@tom.com; franklin67@126.com; Gerhard.Franz@chemie.uni-regensburg.de; glen.hanson@hsc.utah.edu; helferic@illinois.edu; herbcowboy@aol.com; hlata@olemiss.edu; ikhan@olemiss.edu; imiele@ci.umsmed.edu; iqbal.choudhary@iccs.edu; jcurtis_bookwalter@agilent.com; jdmccchesney@yahoo.com; jelowe@olemiss.edu; jhcaddellina@aol.com; jianping@olemiss.edu; jnnfryt@olemiss.edu; jyang1@olemiss.edu; jzhang3@olemiss.edu; Kate_Yu@waters.com; khcbaser@anadolu.edu.tr; kims@snu.ac.kr; lijuan@mail.shcnc.ac.cn; loren@LDIGroup.com; lpgin@smmu.edu.cn; lspears@olemiss.edu; LubahnD@missouri.edu; lwalker@olemiss.edu; lykong@jlonline.com; lyzhong@olemiss.edu; machan@olemiss.edu; mazaki@olemiss.edu; melwang@olemiss.edu; melsohly@olemiss.edu; mhharon@olemiss.edu; michael.eason@wildflower.org; mjcunnin@olemiss.edu; mkashfaq@olemiss.edu; mmbenn@olemiss.edu; mradwan@olemiss.edu; msabdelb@olemiss.edu; mtantry@olemiss.edu; ndchaura@olemiss.edu; nhtan@mail.kib.ac.cn; nimish@charak.com; ntechen@olemiss.edu; okunjic@niaid.nih.gov; paula_brown@bcit.ca; pharm.wiss@uni-graz.at; pharmgao@tju.edu.cn; phdranga@olemiss.edu; pnm245@yahoo.com; prembala@olemiss.edu; quxiabobo0504@hotmail.com; rap@sdindia.com; rcswamy@olemiss.edu; rford@olemiss.edu; rimikell@olemiss.edu; Rkingston@safetycall.com; ravu@olemiss.edu; rwdharma@olemiss.edu; sahopper@olemiss.edu; sbsandu@sandu.in; schilton@wfbmc.edu; Sdentali@ahpa.org; sehrawat_r@yahoo.com; shigen@inm.u-toyama.ac.jp; simhmuhl@mail.shcnc.ac.cn; sjain@olemiss.edu; sjpate@email.wcu.edu; skhan@olemiss.edu; spmanly@olemiss.edu; ssross@olemiss.edu; srweeras@olemiss.edu; suman@olemiss.edu; sydspain@olemiss.edu; tflastersprint@earthlink.net; TOUWAIDA@SI.EDU; travis@nsf.org; tsmillie@olemiss.edu; vcrsp@olemiss.edu; viseshayurved@gmail.com; voxa@olemiss.edu; vradelli@olemiss.edu; vraman@olemiss.edu; [wangwei@olemiss.edu](mailto>wangwei@olemiss.edu); wangyh@olemiss.edu; wbla@pharmazie.uni-kiel.de; wchambli@olemiss.edu; Wendy.applequist@mobot.org; wgul@elsohly.com; wherath@olemiss.edu; William.Cefalu@pbrc.edu; xcli7@olemiss.edu; xyj6492@sohu.com; yqding@olemiss.edu; yve@mail.shcnc.ac.cn; zmedic@olemiss.edu; zulfiqar@olemiss.edu; allison.richard@pbrc.edu; ljs@bastyr.edu; kaettefa@uncg.edu; mikumari@olemiss.edu; pauldurham@missouristate.edu; nakanohr@affrc.go.jp; phil.brantley@pbrc.edu; meriem.ouchfoun@usherbrooke.ca; alcerdei@olemiss.edu; gwc@bentcreekinstitute.org; xfu2@olemiss.edu; dnagle@olemiss.edu; Ali_Akhtar; ntabanca@olemiss.edu; dmcheng@rci.rutgers.edu; zhiquang.pan@ars.usda.gov; ordale@olemiss.edu; gphobby@uams.edu; peter.scherp@pbrc.edu; jmh@bruker.com; rsriniv@gmail.com; sduke@olemiss.edu; ahtarawn@olemiss.edu; giovanni.appendino@indena.com; jiith@gencorpacific.com; dnelinson@naturesvalue.com; asok@olemiss.edu; diana.obanda@pbrc.edu; info@bentcreekinstitute.org; clcantr1@olemiss.edu; Pawar, Rahul; afeefhusni@gmail.com; sml@bruker.com; ynvsque@olemiss.edu; ndpugh@olemiss.edu; chufford@olemiss.edu; a.bily@naturex.com; helmi.hussien@hc-sc.gc.ca; gombae360@hanmail.net; Hazrah_moothoo@bcit.ca; steve@newhope.com; melissa.phillips@nist.gov; Tamta, Hemlata; mike.visnick@reatapharma.com; mhs@usp.org; harry@unpa.com; syleshv@chromadex.com; dwedge@olemiss.edu; brian.schaneberg@mss.affem.com; harvey.punia@hc-sc.gc.ca; tarifg@naturesvalue.com; Greenhaw, James; Yang, Xi; ray.cooper@novusint.com; anavarrt@unam.mx; gasher@med.unc.edu; michael.lelah@nowfoods.com; shahid.perwaiz@hc-sc.gc.ca; stefano.togni@indena.com; malindra@nbc.lk; emsaleh@olemiss.edu; cassy.quave@gmail.com; jli@olemiss.edu; alessandra.storzini@indena.com; riveroic@yahoo.com.mx; john.balles@nutrilite.com; 26niols@bellsouth.net; prpolepa@olemiss.edu; gbyi@olemiss.edu; zhushu73@gmail.com
Subject: Our Thanks
Date: Monday, April 18, 2011 10:01:25 AM

Dear Friends,

Now that the 10th anniversary meeting of the ICSB has come to an end, we would like to express our sincere gratitude to all of our speakers, organizers, sponsors, exhibitors, staff, volunteers and every attendee. It is only through your support and participation that makes this conference an overwhelming success. We hope that you enjoyed the conference and all its events, and found the presentations and panels informative and interesting. Next year's conference is set for April 16th – 19th, 2012, and we hope to see you all again. With your support, the ICSB will continue to grow and improve long into the future. Feel free to send any questions/suggestions for the upcoming conference to ICSB@olemiss.edu, and once again thank you for participating in the 10th Oxford International Conference on the Science of Botanicals.

Regards,
Ikhlās Khan,
Troy Smillie and
the ICSB Team

From: Ikhlas A. Khan
To: [Pawar, Rahul](#)
Subject: Out of the office (was Re: Request for a reco letter)
Date: Friday, June 3, 2011 11:20:18 AM

I am currently out of the office till June 3rd. If you need immediate assistance please contact Mrs. Jennifer Taylor (662) 915-1090, jnnfrtyl@olemiss.edu or Dr. Troy Smillie (662) 915-1168, tsmillie@olemiss.edu.

--

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: paper
Date: Monday, June 8, 2015 2:41:19 PM

could U please mail me following paper:

Bharathi Avula, Yan-Hong Wang, Ikhlas A. Khan. Arsenic speciation and fucoxanthin analysis from seaweed dietary supplements using LC-MS. Journal of AOAC International (2015), 98(2), 321-329.

thanks
bharathi

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: Paper request
Date: Friday, November 18, 2011 2:15:15 PM

Could you email me the following paper:

Quantitative Determination of Pregnanes from Aerial Parts of *Caralluma* Species Using HPLC-UV and Identification by LC-ESI-TOF: [Journal of AOAC International](#), Volume 94, Number 5, September 2011 , pp. 1383-1390(8)

Have a good weekend

Thank you

Bharathi

From: bavula@olemiss.edu
To: [Pawar, Rahul](#)
Subject: Paper request
Date: Friday, January 28, 2011 9:19:38 AM

Could you please send me the following paper:

A field guide to instrumentation

By Anon.

From Journal of AOAC International (2009), 92(6), 205A-213A.

Thank you
Bharathi

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: paper request
Date: Tuesday, May 13, 2014 10:13:31 AM

Could you please mail me the following paper:

[J AOAC Int.](#) 2010 May-Jun;93(3):849-54. Microwave-assisted extraction coupled with single drop microextraction and high-performance column liquid chromatography for the determination of trace estrogen adulterants in soybean isoflavone dietary supplements. [Xiao X](#)¹, [Yin Y](#), [Hu Y](#), [Li G](#).

Thank you
bharathi

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: paper request
Date: Wednesday, March 13, 2013 9:39:10 AM

Could you please email me the following paper:

[Rapid High-Performance Thin-Layer Chromatographic Method for Detection of 5 Adulteration of Black Cohosh with *Cimicifuga foetida*, *C. heracleifolia*, *C. dahurica*, or *C. americana*](#). Authors: Ankli, Anita; Reich, Eike; Steiner, Mario

Source: **[Journal of AOAC International](#)**, Volume 91, Number 6, November 2008 , pp. 1257-1264(8)

Thank you
Bharathi

From: bavula@olemiss.edu
To: [""Pawar; Pawar, Rahul](#)
Subject: Paper Request
Date: Thursday, January 28, 2010 2:37:40 PM

Could you please mail me the following paper:



Simultaneous Quantification of Adrenergic Amines and Flavonoids in *C. aurantium*, various *Citrus* species and Dietary Supplements by Liquid Chromatography. *Journal of AOAC International* (2005), 88(6), 1593-1606.

Also could you please also send me the sample letters for EB-1

Thank you
Bharathi

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: paper
Date: Thursday, July 25, 2013 2:48:49 PM

Could you please mail me the following paper:

[Synchronous high-performance liquid chromatography with a photodiode array detector and mass spectrometry for the determination of citrinin, monascin, ankaflavin, and the lactone and acid forms of monacolin K in red mold rice.](#) Wu, Cheng-Lun; Kuo, Yao-Haur; Lee, Chun-Lin; Hsu, Ya-Wen; Pan, Tzu-Ming. Journal of AOAC International (2011), 94(1), 179-190.

Thank you
bharathi

From: bavula@olemiss.edu
To: [Pawar, Rahul](#)
Subject: Papers request
Date: Tuesday, July 20, 2010 9:16:19 AM

Could you please email me following papers:

Determination of campesterol, stigmasterol, and beta-sitosterol in saw palmetto raw materials and dietary supplements by gas chromatography: single-laboratory validation. Sorenson, Wendy R.; Sullivan, Darryl. Covance Laboratories, Madison, WI, USA. Journal of AOAC International (2006), 89(1), 22-34.

Determination of campesterol, stigmasterol, and beta-sitosterol in saw palmetto raw materials and dietary supplements by gas chromatography: collaborative study. Sorenson, Wendy R.; Sullivan, Darryl. Covance Laboratories, Madison, WI, USA. Journal of AOAC International (2007),

90(3), 670-678.

I hope you enjoyed your trip

**Thank you
Bharathi**

From: [Pawar, Rahul](#)
To: [SATYANARAYANARAJU SAGI](#)
Subject: presentation
Date: Tuesday, June 21, 2016 11:27:00 PM

Send me your slides by email so I can have them ready for presentation.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Tuesday, June 21, 2016 6:56 PM
To: Pawar, Rahul
Subject: Re: Ticket confirmed

Hi Rahul,
Due to bad in DC, I'm have terrible flight journal, two hours flight stayed on Memphis runway. Now it landed in Columbus, Ohio. I will update flight status.

Thanks

Satyanarayanaraju Sagi

On Jun 21, 2016, at 10:04 AM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov> wrote:

Thanks Raju,
Tomorrow when you arrive at the office lobby, after the security check, ask the Security guards to call me (240-402-1795) so I will then escort you in. Do carry your ID as it needed for making the visitors badge.
Check if your hotel provides free shuttle to the College Park metro station. FDA is right in front of the metro station. And do let me know if you need any help.
Best,
Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Monday, June 20, 2016 3:20 PM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

Hi Rahul,

Thanks for the information.
Here is my Bio (made it very simple).

Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Monday, June 20, 2016 1:39 PM

To: SATYANARAYANARAJU SAGI
Subject: RE: Ticket confirmed

Hi Raju,
This is your schedule, if there are any changes I will let you know.
Also, send me your bio for introduction.
Thanks,
Rahul

Schedule for Dr. Sagi

9:00 AM: Dr. Sagi's arrival: Rahul's Office
9: 30-9:45 Meeting with Dr. Cynthia Srigley
9:45-10:15 AM: Lab tour and meeting with other Division members.
10:30 to 11.30 AM: Dr. Sagi's presentation and Q&A
11:45-12:15 PM: Meeting with Dr. Noonan
12:15- 1:30 PM: Lunch
1:30- 2:00 PM: Meeting with Dr. Shaun MacMahon
2:00-2:30 PM: Meeting with Dr. Betsy Yakes
2: 30-3:00: Meeting with Dr. James Wittenberg and Dr. Rakhi Panda
3:00- 3:30: Meeting with Dr: Perry Wang
3:30 PM: Departure

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Monday, June 20, 2016 10:53 AM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

Hi Rahul,

No, I'm Non-vegetarian.
Did u get any agenda? Can you please provide a rough schedule for Wednesday.
I will start around 9:30AM from oxford and reach DC by 4:30PM.
I will call you once I reach my hotel.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Monday, June 20, 2016 9:43 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Ticket confirmed

Raju, are you vegetarian?

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]

Sent: Friday, June 17, 2016 9:03 AM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

OK Rahul

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Thursday, June 16, 2016 11:37 PM
To: SATYANARAYANARAJU SAGI
Subject: RE: Ticket confirmed

Hi Raju, Will let you know next week. Best, Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Thursday, June 16, 2016 5:49 PM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

Hi Rahul,
Just a reminder, have you got any agenda for my interview??

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Thursday, June 09, 2016 10:27 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Ticket confirmed

You are good, job description is pretty much what you do at Umiss. Development and validation of analytical methods. Currently, we have minor interest in natural product isolation but characterization is very relevant. If I get a formal description I may provide. Hope this helps.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Thursday, June 09, 2016 11:22 AM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

Hi Rahul,
If I have job description, I can prepare for interview with group members accordingly.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]

Sent: Thursday, June 09, 2016 10:09 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Ticket confirmed

Can you tell me why you need the job description?

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Thursday, June 09, 2016 9:42 AM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

Hi Rahul,
I'm working on presentation. I'm supposed to ask for agenda. If you have some free time and can you send "Job description".

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Wednesday, June 08, 2016 10:12 PM
To: SATYANARAYANARAJU SAGI
Subject: RE: Ticket confirmed

Hi Raju,
That's, good, things are falling in place.
Have you started to prepare a presentation? Be prepared to talk for 30-40 mins and 10-15 mins for questions. I will send final agenda soon.
Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Wednesday, June 08, 2016 5:44 PM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

Hi Rahul,

Hotel reservation confirmed and details are here

Clarion Inn
8601 Baltimore Avenue,
College Park, MD, US, 20740
[+1 \(301\) 474-2800](tel:+13014742800)

One night stay on 21st June (Check in 21st June and Check out 22nd June)

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Tuesday, June 07, 2016 2:10 PM

To: SATYANARAYANARAJU SAGI
Subject: RE: Ticket confirmed

Great, Have you informed Bharathi and Dr. Khan about your interview?

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Tuesday, June 07, 2016 9:41 AM
To: Pawar, Rahul
Subject: Ticket confirmed

Hi Rahul,

Please find the my itinerary.

Will let you know about my lodging once I book my hotel

Thanks
Raju

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Re:
Date: Wednesday, August 29, 2012 5:17:51 PM

I asked Aruna to send some pictures to you. I will arrive on Thursday the 6th, Jennifer will let you know my itinerary. It will be nice if you can pick me up the morning of 7th . Have safe and fun trip.
Ik

From: Rahul Pawar <Rahul.Pawar@fda.hhs.gov>
Date: Wed, 29 Aug 2012 21:03:39 +0000
To: Ikhlas Khan <ikh@olemiss.edu>

Hello Dr. Khan,
I hope you all fine and also hope the storm treated you well.
I am asking for a favor. We were writing a review article covering the topics on Momordica charantia, Stevia, Monk fruit ([Siraitia grosvenorii](#)), Geranium and Blue Cohosh and were wondering if you could provide us some color pictures to include in the review.
Please don't mind if I am unable to respond to your e-mails as I will be out of office for next four days
(b) (6) and will not have access to the office e-mail.
Hope to see you in a while and let me know if you need any help during your trip to DC. Thanks
Best regards
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: [SATYANARAYANARAJU SAGI](#)
Subject: RE:
Date: Monday, June 6, 2016 3:21:00 PM

Ok..

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Monday, June 06, 2016 3:21 PM
To: Pawar, Rahul
Subject: RE:

Got mail from ORISE person stated the fund approval, rules for reimbursement, suppose to send travel flight details for my approval.

Thanks
Sagi

From: Pawar, Rahul [Rahul.Pawar@fda.hhs.gov]
Sent: Monday, June 06, 2016 8:15 AM
To: SATYANARAYANARAJU SAGI
Subject: RE:

Me too. Don't worry, it is their responsibility to arrange for your travel. The interview date should not change.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Monday, June 06, 2016 9:02 AM
To: Pawar, Rahul
Subject: RE:

Hi Rahul,
I didn't hear anything from ORISE people.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Tuesday, May 31, 2016 7:41 AM
To: SATYANARAYANARAJU SAGI
Subject: RE:

Raju,
Please keep me posted on your communications with ORISE people.
Thanks,
Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Wednesday, May 25, 2016 9:10 AM

To: Pawar, Rahul
Subject: RE:

I'm sorry forgot to attach the file, here is the file

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Wednesday, May 25, 2016 8:08 AM
To: SATYANARAYANARAJU SAGI
Subject: RE:

To begin I need the file!

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Wednesday, May 25, 2016 9:07 AM
To: Pawar, Rahul
Subject: RE:

Hi Rahul,

Please find the enclosed file. Let me know if you require any information.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Wednesday, May 25, 2016 7:29 AM
To: SATYANARAYANARAJU SAGI
Subject:

Raju,

Can you please fill out this form, as much as you can, and send it back to me.

Roughly, you can reserve time from 9 to 3 pm on 22nd June for the presentation, interview, lunch and meeting here.

You can choose travel to any Washington airports (IAD, DCA, BWI) and then take a shuttle, taxi or metro to reach a hotel in College Park or Beltsville MD area.

Let me know if you need more information.

Thanks,
Rahul

From: [Pawar, Rahul](#)
To: [SATYANARAYANARAJU SAGI](#)
Subject: RE:
Date: Monday, June 6, 2016 9:15:00 AM

Me too. Don't worry, it is their responsibility to arrange for your travel. The interview date should not change.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Monday, June 06, 2016 9:02 AM
To: Pawar, Rahul
Subject: RE:

Hi Rahul,
I didn't hear anything from ORISE people.

Thanks
Raju

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Rahul

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You can choose travel to any Washington airports (IAD, DCA, BWI) and then take a shuttle, taxi or metro to reach a hotel in College Park or Beltsville MD area.

Let me know if you need more information.

Thanks,
Rahul

From: SATYANARAYANARAJU SAGI
To: [Pawar, Rahul](#)
Subject: RE:
Date: Monday, June 6, 2016 9:02:12 AM

Hi Rahul,
I didn't hear anything from ORISE people.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
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Thanks

Raju

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Sent: Wednesday, May 25, 2016 7:29 AM

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Subject:

Raju,

Can you please fill out this form, as much as you can, and send it back to me.

Roughly, you can reserve time from 9 to 3 pm on 22nd June for the presentation, interview, lunch and meeting here.

You can choose travel to any Washington airports (IAD, DCA, BWI) and then take a shuttle, taxi or metro to reach a hotel in College Park or Beltsville MD area.

Let me know if you need more information.

Thanks,

Rahul

From: [Pawar, Rahul](#)
To: [SATYANARAYANARAJU SAGI](#)
Subject: RE:
Date: Tuesday, May 31, 2016 8:41:00 AM

Raju,
Please keep me posted on your communications with ORISE people.
Thanks,
Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Wednesday, May 25, 2016 9:10 AM
To: Pawar, Rahul
Subject: RE:

I'm sorry forgot to attach the file, here is the file

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Wednesday, May 25, 2016 8:08 AM
To: SATYANARAYANARAJU SAGI
Subject: RE:

To begin I need the file!

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Wednesday, May 25, 2016 9:07 AM
To: Pawar, Rahul
Subject: RE:

Hi Rahul,

Please find the enclosed file. Let me know if you require any information.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Wednesday, May 25, 2016 7:29 AM
To: SATYANARAYANARAJU SAGI
Subject:

Raju,
Can you please fill out this form, as much as you can, and send it back to me.
Roughly, you can reserve time from 9 to 3 pm on 22nd June for the presentation, interview, lunch and meeting here.

You can choose travel to any Washington airports (IAD, DCA, BWI) and then take a shuttle, taxi or metro to reach a hotel in College Park or Beltsville MD area.

Let me know if you need more information.

Thanks,

Rahul

From: SATYANARAYANARAJU SAGI
To: [Pawar, Rahul](#)
Subject: RE:
Date: Wednesday, May 25, 2016 9:09:36 AM
Attachments: [Sagi Interview Travel Form EF.pdf](#)

I'm sorry forgot to attach the file, here is the file

Thanks
Raju

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Wednesday, May 25, 2016 8:08 AM
To: SATYANARAYANARAJU SAGI
Subject: RE:

To begin I need the file!

From: SATYANARAYANARAJU SAGI [mailto:ssagi@olemiss.edu]
Sent: Wednesday, May 25, 2016 9:07 AM
To: Pawar, Rahul
Subject: RE:

Hi Rahul,

Please find the enclosed file. Let me know if you require any information.

Thanks
Raju

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Wednesday, May 25, 2016 7:29 AM
To: SATYANARAYANARAJU SAGI
Subject:

Raju,

Can you please fill out this form, as much as you can, and send it back to me.

Roughly, you can reserve time from 9 to 3 pm on 22nd June for the presentation, interview, lunch and meeting here.

You can choose travel to any Washington airports (IAD, DCA, BWI) and then take a shuttle, taxi or metro to reach a hotel in College Park or Beltsville MD area.

Let me know if you need more information.

Thanks,
Rahul

From: [Pawar, Rahul](#)
To: [SATYANARAYANARAJU SAGI](#)
Subject: RE:
Date: Wednesday, May 25, 2016 9:08:00 AM

To begin I need the file!

From: SATYANARAYANARAJU SAGI [mailto:ssagi@olemiss.edu]
Sent: Wednesday, May 25, 2016 9:07 AM
To: Pawar, Rahul
Subject: RE:

Hi Rahul,

Please find the enclosed file. Let me know if you require any information.

Thanks
Raju

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Wednesday, May 25, 2016 7:29 AM
To: SATYANARAYANARAJU SAGI
Subject:

Raju,

Can you please fill out this form, as much as you can, and send it back to me.

Roughly, you can reserve time from 9 to 3 pm on 22nd June for the presentation, interview, lunch and meeting here.

You can choose travel to any Washington airports (IAD, DCA, BWI) and then take a shuttle, taxi or metro to reach a hotel in College Park or Beltsville MD area.

Let me know if you need more information.

Thanks,
Rahul

From: SATYANARAYANARAJU SAGI
To: [Pawar, Rahul](#)
Subject: RE:
Date: Wednesday, May 25, 2016 9:07:33 AM

Hi Rahul,

Please find the enclosed file. Let me know if you require any information.

Thanks

Raju

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Wednesday, May 25, 2016 7:29 AM
To: SATYANARAYANARAJU SAGI
Subject:

Raju,

Can you please fill out this form, as much as you can, and send it back to me.

Roughly, you can reserve time from 9 to 3 pm on 22nd June for the presentation, interview, lunch and meeting here.

You can choose travel to any Washington airports (IAD, DCA, BWI) and then take a shuttle, taxi or metro to reach a hotel in College Park or Beltsville MD area.

Let me know if you need more information.

Thanks,

Rahul

From: SATYANARAYANARAJU SAGI
To: [Pawar, Rahul](#)
Subject: RE:
Date: Wednesday, May 25, 2016 8:50:26 AM

Hi Rahul,

Can I have your contact number please, I want to talk to you.

Thanks

Raju

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Wednesday, May 25, 2016 7:29 AM
To: SATYANARAYANARAJU SAGI
Subject:

Raju,

Can you please fill out this form, as much as you can, and send it back to me.

Roughly, you can reserve time from 9 to 3 pm on 22nd June for the presentation, interview, lunch and meeting here.

You can choose travel to any Washington airports (IAD, DCA, BWI) and then take a shuttle, taxi or metro to reach a hotel in College Park or Beltsville MD area.

Let me know if you need more information.

Thanks,

Rahul

From: [Pawar, Rahul](#)
To: [SATYANARAYANARAJU SAGI](#)
Subject: RE:
Date: Wednesday, May 25, 2016 8:50:00 AM

240-402-1795

From: SATYANARAYANARAJU SAGI [mailto:ssagi@olemiss.edu]
Sent: Wednesday, May 25, 2016 8:50 AM
To: Pawar, Rahul
Subject: RE:

Hi Rahul,
Can I have your contact number please, I want to talk to you.

Thanks
Raju

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Wednesday, May 25, 2016 7:29 AM
To: SATYANARAYANARAJU SAGI
Subject:

Raju,
Can you please fill out this form, as much as you can, and send it back to me.
Roughly, you can reserve time from 9 to 3 pm on 22nd June for the presentation, interview, lunch and meeting here.
You can choose travel to any Washington airports (IAD, DCA, BWI) and then take a shuttle, taxi or metro to reach a hotel in College Park or Beltsville MD area.
Let me know if you need more information.
Thanks,
Rahul

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: RE:
Date: Thursday, July 25, 2013 3:52:05 PM

Thank you Rahul

bharathi

From: Pawar, Rahul [Rahul.Pawar@fda.hhs.gov]
Sent: Thursday, July 25, 2013 2:14 PM
To: BHARATHI AVULA
Subject:

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: RE:
Date: Tuesday, July 23, 2013 12:54:01 PM

Thank you Rahul

bharathi

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Tuesday, July 23, 2013 11:44 AM
To: BHARATHI AVULA
Subject:

From: [Pawar, Rahul](#)
To: ["Aruna Weerasooriya"](#)
Subject: RE:
Date: Sunday, September 16, 2012 8:36:37 AM

Hi Aruna,

Great to hear from you. That is ok, I could download one cohosh picture from NY bot garden. I was little worried about the monk fruit. Any idea where I can get one from and if it is ok to use Wikipedia pictures.

Your bosses were here on here in our office, had a lunch with them on Friday.

I know you are overstretched and sure you are the best one to handle it.

Take care and convey our regards to girls.

Rahul

From: Aruna Weerasooriya [mailto:arunaw@olemiss.edu]
Sent: Friday, September 14, 2012 1:20 PM
To: Pawar, Rahul
Subject:

Dear Rahul,

Sorry for the delay in getting back to you. Today only I saw your voice mail because the telephone and internet system in the new building had some issues and all of a sudden I started getting all voice mails from the server. Anyway, We do not have any plants or pictures of Blue Cohosh. I actually have nothing to do with that DC conference. The organizers have put my name there without even asking but I have no time for these things! Things are very hectic here without enough staff and I am very near to give this up!!!

Best,

Aruna

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE:
Date: Wednesday, September 5, 2012 6:13:34 PM

Ok, talk to you tomorrow.

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Wednesday, September 05, 2012 5:11 PM
To: Pawar, Rahul
Subject: Re:

It might be too much for you. I can take Taxi and inform you once I reached.
Thanks
IK

From: Rahul Pawar <Rahul.Pawar@fda.hhs.gov>
Date: Wed, 5 Sep 2012 18:33:09 +0000
To: Ikhlas Khan <ikhan@olemiss.edu>
Subject: RE:

Hello Dr. Khan,
Aruna send me some pictures today, thank you.
I will pick you up from the hotel on 7th morning from the hotel and I can also pick you up tomorrow from the airport.
Regards
Rahul

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Wednesday, August 29, 2012 5:18 PM
To: Pawar, Rahul
Subject: Re:

I asked Aruna to send some pictures to you. I will arrive on Thursday the 6th, Jennifer will let you know my itinerary. It will be nice if you can pick me up the morning of 7th .
Have safe and fun trip.
Ik

From: Rahul Pawar <Rahul.Pawar@fda.hhs.gov>
Date: Wed, 29 Aug 2012 21:03:39 +0000
To: Ikhlas Khan <ikhan@olemiss.edu>

Hello Dr. Khan,
I hope you all fine and also hope the storm treated you well.
I am asking for a favor. We were writing a review article covering the topics on Momordica charantia, Stevia, Monk fruit ([Siraitia grosvenorii](#)), Geranium and Blue Cohosh and were wondering if you could provide us some color pictures to include in the review.

Please don't mind if I am unable to respond to your e-mails as I will be out of office for next four days

(b) (6)!) and will not have access to the office e-mail.

Hope to see you in a while and let me know if you need any help during your trip to DC. Thanks

Best regards

Rahul

Rahul Pawar Ph.D.

Division of Bioanalytical Chemistry

CFSAN-FDA

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 240-402-1795

Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE:
Date: Wednesday, September 5, 2012 2:33:09 PM

Hello Dr. Khan,
Aruna send me some pictures today, thank you.
I will pick you up from the hotel on 7th morning from the hotel and I can also pick you up tomorrow from the airport.
Regards
Rahul

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Wednesday, August 29, 2012 5:18 PM
To: Pawar, Rahul
Subject: Re:

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From: Rahul Pawar <Rahul.Pawar@fda.hhs.gov>
Date: Wed, 29 Aug 2012 21:03:39 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>

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CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE:
Date: Wednesday, August 29, 2012 5:22:19 PM

Thank you Sir, If you don't have any plans then we can head out for dinner nearby.
See you soon
Rahul

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Wednesday, August 29, 2012 5:18 PM
To: Pawar, Rahul
Subject: Re:

I asked Aruna to send some pictures to you. I will arrive on Thursday the 6th, Jennifer will let you know my itinerary. It will be nice if you can pick me up the morning of 7th .
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From: Rahul Pawar <Rahul.Pawar@fda.hhs.gov>
Date: Wed, 29 Aug 2012 21:03:39 +0000
To: Ikhlas Khan <ikhan@olemiss.edu>

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Division of Bioanalytical Chemistry
CFSA-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: Ikhlas Khan
To: Pawar_Rahul
Subject: RE:
Date: Tuesday, August 14, 2012 3:15:12 PM

Hi Rahul

Please forward CFSAN email.

IK

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Tuesday, August 14, 2012 1:41 PM
To: Ikhlas Khan
Subject: RE:

http://www.separationsnow.com/details/ezine/2459741/Hide_and_seek_Controversial_natural_stimulant_in_sports_supplements_is_synthetic.html

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Monday, August 13, 2012 9:38 AM
To: Pawar, Rahul; BHARATHI AVULA
Subject: RE:

Hi Rahul

It was great to see you and family. Yes we not only saw it we have to deal with it. Its paid research and manuscript was written by lawyers.

IK

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Monday, August 13, 2012 8:28 AM
To: Ikhlas Khan; BHARATHI AVULA
Subject:

Good morning,
Hope you all are doing well, It was wonderful to see you at NY.
I hope you guys saw this recent study
Best regards
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: Pawar, Rahul
To: "Ikhlas Khan"
Subject: RE:
Date: Tuesday, August 14, 2012 2:40:33 PM

http://www.separationsnow.com/details/ezine/2459741/Hide_and_seek_Controversial_natural_stimulant_in_sports_supplements_is_synthetic.html

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Monday, August 13, 2012 9:38 AM
To: Pawar, Rahul; BHARATHI AVULA
Subject: RE:

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College Park, MD 20740
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Fax: 301-436-2622

From: Ikhlas Khan
To: [Pawar, Rahul](#); [BHARATHI AVULA](#)
Subject: RE:
Date: Monday, August 13, 2012 9:38:34 AM

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Best regards
Rahul

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Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE:
Date: Monday, May 14, 2012 9:19:00 AM

Good morning Dr. Khan,
I spoke with Jeanne last week regarding the Acacia work. She said she will talk with you regarding this. I also spoke with my supervisor (Alex Krynitsky) regarding the possible job opening. He mentioned that only Citizens will be eligible to apply for the current position. But I will look further and let you and Wang know.
Best regards
Rahul

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Tuesday, May 08, 2012 5:55 PM
To: Pawar, Rahul
Subject: <no subject>

Dear Rahul

How are you. I know you are working on Acacia rigidula and found biogenic amines. I have been asked by Fabricant to do animal behavioral studies on it. Just taking extract of acacia might not help since they are minor compounds. I was wondering if you can provide extract enriched with biogenic amines we can do animal study.

Let me know what you think before you or I talk to Jeanne. Call me if you can
IK

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: RE:
Date: Friday, November 18, 2011 2:26:21 PM

Thank you Rahul

Bharathi

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Friday, November 18, 2011 1:24 PM
To: BHARATHI AVULA
Subject:

Hello, have a good weekend
tty soon

From: [Pawar, Rahul](#)
To: ["Ikhlas A. Khan"](#)
Subject: RE:
Date: Wednesday, August 25, 2010 5:04:00 PM

Thanks, that is ok

-----Original Message-----

From: Ikhlas A. Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Wednesday, August 25, 2010 4:49 PM
To: Pawar, Rahul
Subject: RE:
Importance: High

I was about to write to you. I will suggest that you pick me up in the morning and in the evening we can go for dinner and don't worry about tomorrow.

My cell# **(b) (6)**
IK

At 03:33 PM 8/25/2010, you wrote:

>Hello Dr Khan,
>If you don't have any other plans then I can pick you up from your
>hotel for dinner tomorrow (Any time after your fast is over).
>Please let me know if will be convenient for you. Also let me know
>the cell phone number you will be carrying with you.
>Rahul
>

>-----Original Message-----

>From: Ikhlas A. Khan [<mailto:ikhlan@olemiss.edu>]
>Sent: Monday, August 23, 2010 5:32 PM
>To: Pawar, Rahul
>Subject: RE:
>
>Thanks.
>IK

>At 03:39 PM 8/23/2010, you wrote:

> >Hi Dr Khan,
> >There was one interview today and the guy was King Lee and he talked
> >about vitamins. There was no announcement as such to the division so
> >I could not attend the talk but by the way heard that it was very general.
> >Best regards
> >Rahul
> >
> >

> >-----Original Message-----

> >From: Ikhlas A. Khan [<mailto:ikhlan@olemiss.edu>]
> >Sent: Thursday, August 19, 2010 12:36 PM
> >To: Pawar, Rahul
> >Subject:
> >
> >Hi Rahul
> >
> >Just to let you know, I am flying on 26th and should be arriving at
> >Washington National at 5:48 P.M.

>> My hotel has been booked, Greenbelt Marriott 6400 Ivy Lane,
>>Greenbelt, Maryland 20770 USA Phone: 1-301-441-3700. I will be flying
>>back on Saturday morning at 11:35 a.m.

>

>Ikhlas A. Khan Ph.D.
>Assistant Director
>Director of FDA Program
>Research Professor and
>Professor, Department of Pharmacognosy
>National Center for Natural Products Research
>School of Pharmacy, University of Mississippi
>MS 38677
>Tel# 662 915 7821
>Fax# 662 915 7989

Ikhlas A. Khan Ph.D.
Assistant Director
Director of FDA Program
Research Professor and
Professor, Department of Pharmacognosy
National Center for Natural Products Research
School of Pharmacy, University of Mississippi
MS 38677
Tel# 662 915 7821
Fax# 662 915 7989

From: [Pawar, Rahul](#)
To: ["Premalatha Balachandran"](#)
Subject: RE:
Date: Tuesday, May 25, 2010 11:43:09 AM

Hi Premalatha,
Weather is too soggy this year, but its comfortable till now.
I will mail the check to you soon. Please confirm your address:P.O.Box 8466, University, MS-38677.
Hi to Karthik and keerthin
Take care
Rahul

-----Original Message-----

From: Premalatha Balachandran [<mailto:prembala@olemiss.edu>]
Sent: Monday, May 24, 2010 5:07 PM
To: Pawar, Rahul
Subject: RE:

Hi Rahul,

I hope you had a nice week end.

How is the weather. Here it is too hot.

We renewed our Sam's club business membership for \$35.

Say hi to (b) (6). Hope (b) (6) is doing good.

regards,
Premalatha

--

Premalatha Balachandran, Ph.D.,
National Center for Natural Products Research,
Research Institute of Pharmaceutical Sciences, School of Pharmacy,
University of Mississippi, MS-38677, USA.
Ph.662-915-3463
Fax:662-915-7062
Visit me at : <http://home.olemiss.edu/~prembala>

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From: [Pawar, Rahul](#)
To: ["Premalatha Balachandran"](#)
Subject: RE:
Date: Thursday, May 20, 2010 12:29:23 PM

Hi Premalatha,
That is ok, once you pay let me know how much I have to pay you.
How is your work going on, anything new?
Are you attending ASP this year, we are attending.
thank you
Rahul

From: Premalatha Balachandran [mailto:prembala@olemiss.edu]
Sent: Thursday, May 20, 2010 12:20 PM
To: Pawar, Rahul
Subject: Re:

Hi Rahul,
We are all fine. Hope to hear the same from you all.
Here is the pdf of the paper. But it is a scanned version.
We will be going to sams club this week end or next week and we will pay the membership dues. I will let you know once it is renewed.
Thanks,
Premalatha

Hi Premalatha,
How are you doing?
Do you have pdf for the following paper?

Pawar, R. S., Balachandran, P., Pasco, D. S., and Khan, I. A. 2006. Cytotoxicity studies of triterpenoids from *Akebia trifoliata* and *Clematis ligusticifolia*. *Acta Horticulturae*, 720: 171-178.

Looks like the membership for samsclub is due sometime next month. Do you want me to pay this time?

Thank you
Rahul

--

Premalatha Balachandran, Ph.D.,
National Center for Natural Products Research,
Research Institute of Pharmaceutical Sciences, School of Pharmacy,

University of Mississippi, MS-38677, USA.
Ph.662-915-3463
Fax:662-915-7062
Visit me at : <http://home.olemiss.edu/~prembala>

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From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Re:
Date: Wednesday, September 5, 2012 5:11:29 PM

It might be too much for you. I can take Taxi and inform you once I reached.

Thanks

IK

From: Rahul Pawar <Rahul.Pawar@fda.hhs.gov>
Date: Wed, 5 Sep 2012 18:33:09 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE:

Hello Dr. Khan,

Aruna send me some pictures today, thank you.

I will pick you up from the hotel on 7th morning from the hotel and I can also pick you up tomorrow from the airport.

Regards

Rahul

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Wednesday, August 29, 2012 5:18 PM
To: Pawar, Rahul
Subject: Re:

I asked Aruna to send some pictures to you. I will arrive on Thursday the 6th, Jennifer will let you know my itinerary. It will be nice if you can pick me up the morning of 7th . Have safe and fun trip.

Ik

From: Rahul Pawar <Rahul.Pawar@fda.hhs.gov>
Date: Wed, 29 Aug 2012 21:03:39 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>

Hello Dr. Khan,

I hope you all fine and also hope the storm treated you well.

I am asking for a favor. We were writing a review article covering the topics on Momordica charantia, Stevia, Monk fruit ([Siraitia grosvenorii](#)), Geranium and Blue Cohosh and were wondering if you could provide us some color pictures to include in the review.

Please don't mind if I am unable to respond to your e-mails as I will be out of office for next four days

(b) (6) and will not have access to the office e-mail.

Hope to see you in a while and let me know if you need any help during your trip to DC. Thanks

Best regards

Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry

CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: Pawar, Rahul
To: "Ikhlas Khan"
Subject: RE: <no subject>
Date: Tuesday, July 8, 2014 1:53:00 PM

Yes, other NIPERs is not a good idea.

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Tuesday, July 08, 2014 12:49 PM
To: Pawar, Rahul
Subject: Re: <no subject>

Yes, I don't think she will get position at Mohali. Other NIPERS I am not excited.
IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tue, 8 Jul 2014 14:55:00 +0000
To: Ikhlas Khan <ikhan@olemiss.edu>
Subject: RE: <no subject>

I hope you know by now but I just checked the Niper website. Suma was ranked 1439 in the general category.

http://www.niper.ac.in/2014_jeecombined_updated.pdf

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Monday, July 07, 2014 1:23 PM
To: Pawar, Rahul
Subject: Re: <no subject>

No problem. Hope your trip went well. We can chat when you settle.
Ik

Sent from my iPhone

On Jul 7, 2014, at 11:59 AM, "Pawar, Rahul" <Rahul.Pawar@fda.hhs.gov> wrote:

Hello Sir,
Sorry for the late response, I could not access my office email in India. I just got back to office today.
Let me know if you still need this information?
Thanks and talk to you soon.
Rahul

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Monday, June 16, 2014 9:43 AM
To: Pawar, Rahul
Subject: <no subject>
Importance: High

Hi Rahul

I hope you reached safely. I need your assistance, My Niece qualified for GPAT and now appeared in NIPER test. Now they do NIPER test it means she can get admission in any NIPER and it does not have to be Mohali. I sent an email to Bhutani but no response. Do you still have some connection there.

Her application# (b) (6)

Registration (b) (6)

Her name is (b) (6)

IK

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Re: <no subject>
Date: Monday, February 11, 2013 3:33:50 PM

Thanks, Yes including ex-boss but don't include the BOSS at home otherwise you will be in trouble.

Check with her secretary, we will be printing invitation card for ICSB where he is chief Guest.

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Mon, 11 Feb 2013 20:27:59 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: <no subject>

Including ex-bosses?

According to a recent organizational listing e-mail, Dr Musser is DEPUTY DIRECTOR FOR REGULATORY AFFAIRS.

If this is for official reasons please confirm with my managers.

Thanks, we all are fine. Hope you all are doing fine too. Mississippi was again in news this weekend, Hope all is well in oxford.

Best
Rahul

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Monday, February 11, 2013 3:14 PM
To: Pawar, Rahul
Subject: <no subject>

Hi Rahul

All your bosses are useless. Can you send me Musser's full designation.

Hope you all doing fine.

Ik

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE: <no subject>
Date: Monday, February 11, 2013 4:59:17 PM

Looks like he is also acting center director.
I have contacted somebody for the answer. I will let you know then.

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Monday, February 11, 2013 3:33 PM
To: Pawar, Rahul
Subject: Re: <no subject>

Thanks, Yes including ex-boss but don't include the BOSS at home otherwise you will be in trouble.
Check with her secretary, we will be printing invitation card for ICSB where he is chief Guest.
IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Mon, 11 Feb 2013 20:27:59 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: <no subject>

Including ex-bosses?

According to a recent organizational listing e-mail, Dr Musser is DEPUTY DIRECTOR FOR REGULATORY AFFAIRS.

If this is for official reasons please confirm with my managers.

Thanks, we all are fine. Hope you all are doing fine too. Mississippi was again in news this weekend, Hope all is well in oxford.

Best
Rahul

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Monday, February 11, 2013 3:14 PM
To: Pawar, Rahul
Subject: <no subject>

Hi Rahul
All your bosses are useless. Can you send me Musser's full designation.
Hope you all doing fine.
Ik

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE: <no subject>
Date: Wednesday, August 22, 2012 12:07:12 AM

Thank you Sir, our personal invitation to Dr. Shabana and Saria to visit us.

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Tuesday, August 21, 2012 11:07 PM
To: Pawar, Rahul
Subject: <no subject>

Thanks, I will be coming to attend ceremony and congratulations to you too. I should be arriving on 6th evening and will leave on 7th in the evening.
IK

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE: <no subject>
Date: Wednesday, May 9, 2012 8:50:00 AM

Good morning Sir,
I will call you today morning.
Rahul

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Tuesday, May 08, 2012 5:55 PM
To: Pawar, Rahul
Subject: <no subject>

Dear Rahul

How are you. I know you are working on *Acacia rigidula* and found biogenic amines. I have been asked by Fabricant to do animal behavioral studies on it. Just taking extract of acacia might not help since they are minor compounds. I was wondering if you can provide extract enriched with biogenic amines we can do animal study.

Let me know what you think before you or I talk to Jeanne. Call me if you can
IK

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Re: <no subject>
Date: Tuesday, April 19, 2016 4:08:30 PM

Thanks, Rahul
ik

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tuesday, April 19, 2016 at 1:55 PM
To: Ikhlas Khan <ikh@olemiss.edu>
Subject: RE: <no subject>

Hello Dr. Khan,
Thanks, It was great to be there after a while.

About isopropyloctopamine: See below, this is information that I wrote in a review on PEAs which is in currently press.

" An NDI notification for Betaphrine was filed with FDA in September, 2004.^[121] After reviewing the notification, FDA concluded that Betaphrine was not a dietary ingredient. Rather, it appeared to be a chemically synthesized substance. FDA noted further that while Betaphrine may be synthesized using one or more precursors that are themselves dietary ingredients, the substance such as Betaphrine that is chemically synthesized using such substances as starting materials is not itself a substance defined as a dietary ingredient in 21 U.S.C. 321(ff)(1).^[122] "

[121] Food and Drug Administration. **2004**, Premarket notification for a New Dietary Ingredient: Betaphrine. Available at: <http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt0258-04-SSPF-vol174.pdf>. [29 June 2015].

[122] Food and Drug Administration. **2004**, Response letter: NDI Notification for Betaphrine. Available at: <http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt0258-02-vol174.pdf>. [29 June 2015].

Hope this helps.
Best wishes to all,
Rahul

From: Ikhlas Khan [<mailto:ikh@olemiss.edu>]
Sent: Tuesday, April 19, 2016 2:21 PM
To: Pawar, Rahul
Subject: <no subject>

Hi Rahul
Hope you reached safely and created some news for ICSB, thanks

Do you know the status of "Isopropyloctopamine", looks like NDI was submitted but not sure the status

If you can find out easily that will be great
ik

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE: 2012 CFSAN Honor Awards
Date: Tuesday, August 14, 2012 6:03:30 PM
Attachments: [2012 CFSAN Honor Awards Winners.pdf](#)

Here it is

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Tuesday, August 14, 2012 4:44 PM
To: Pawar, Rahul
Subject: RE: 2012 CFSAN Honor Awards

I think you have to attach pdf separately since its not opening with the link.
ik

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Tuesday, August 14, 2012 3:38 PM
To: Ikhlas Khan
Subject: FW: 2012 CFSAN Honor Awards

From: CFSAN-ALLHANDS
Sent: Monday, August 13, 2012 3:58 PM
To: CFSAN-All Hands
Subject: 2012 CFSAN Honor Awards

To: All CFSAN Employees:

I am pleased to announce that the CFSAN Honor Awards Ceremony will be held on Friday September 7, 2012 in the Wiley Building Auditorium from 11 am – 1 pm with a reception immediately following in the Atrium. This celebration gives CFSAN management a wonderful opportunity to highlight the Center's accomplishments and to honor and recognize the noteworthy contributions of our colleagues.

Phil Spiller, Steve Musser, and I invite you to attend and celebrate with us the exceptional accomplishments of our CFSAN employees.

Congratulations to each one of you for a job well done, and we look forward to seeing all of you at the upcoming CFSAN Awards Ceremony. Attached is a list of the award recipients. If you have any questions concerning the upcoming awards ceremony, please contact [Tanya Gray](#) by email or telephone at 240-402-2952.

Michael M. Landa

2012 CFSAN Honor Award Recipients

(-OM-)

Please note you are receiving this email because you are using an email account supported by the Department of Health and Human Services. Please do not respond to this email. If you have questions or comments regarding this specific message, please write to the responsible party. If you would like to submit a message for CFSAN All-Hands, please email "OM Action".

From: [Pawar, Rahul](#)
To: [Jennifer S. Taylor](#)
Subject: RE: 2016 ICSB
Date: Friday, April 1, 2016 8:09:00 AM

Hi Jennifer,
Here is my itinerary.

1. Flight Baltimore, MD (BWI) to Memphis, TN (MEM)
Southwest 3401,
Departure: 03:15 PM
Arrival: 04:40 PM, Memphis Intl (MEM)

2. Flight Memphis, TN (MEM) to Baltimore, MD (BWI)
Southwest 545
Departure: 04:05 PM
Arrival: 07:10 PM, Baltimore Washington Intl Arpt (BWI)

3. Hampton Inn Oxford Conference Center
103 Ed Perry Blvd.
Oxford, MS, 38655
US
FONE 662-234-5565

Checking In: Sun Apr 10,
Days 5, Guests 1
Checking Out: Fri Apr 15

I have not yet reserved the shuttle but I feel I might need the shuttle for my return journey.

Thanks, see you next week!
Rhaul

From: Jennifer S. Taylor [mailto:jnnfrtyl@olemiss.edu]
Sent: Thursday, March 31, 2016 5:32 PM
To: Pawar, Rahul
Subject: 2016 ICSB

Rahul, we are looking forward to seeing you in a couple weeks. If you have finalized your itinerary please send it to me and also let me know if you will need the shuttle service. Let me know if you need any assistance in your preparations.

Sincerely,

Jennifer Taylor

Jennifer Taylor

Program Coordinator

University of Mississippi

National Center for Natural Products Research

3012 Thad Cochran

P.O. Box 1848

University, MS 38677

✉ jnnfrtyl@olemiss.edu

☎ 662-915-1090

📠 662-915-7989

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From: YANHONG WANG
To: [Pawar, Rahul](#)
Subject: RE: about FDA position
Date: Monday, May 14, 2012 10:06:58 AM

Dear Rahul,

Good morning!

My cell phone number is (b) (6) Please help me to keep eyes open on any future opportunity.

Thank you!

Yan-Hong Wang

From: Pawar, Rahul [Rahul.Pawar@fda.hhs.gov]
Sent: Monday, May 14, 2012 8:25 AM
To: YANHONG WANG
Subject: RE: about FDA position

Dear Dr. Wang,

Not a problem. Mean time, I also spoke with my supervisor (Alex Krynitsky) regarding the possible job opening. He mentioned that only citizens are eligible to apply for the current position. But I will look further and let you know.

What will be the best phone number to call you if I need to?

Best regards

Rahul

From: YANHONG WANG [mailto:wangyh@olemiss.edu]
Sent: Friday, May 11, 2012 5:35 PM
To: Pawar, Rahul
Subject: RE: about FDA position

Dear Rahul,

Sorry for mistaking to type your name in last email.

Wish you and your family have a wonderful weekend!

Yan-Hong Wang

From: Pawar, Rahul [Rahul.Pawar@fda.hhs.gov]
Sent: Friday, May 11, 2012 10:23 AM
To: YANHONG WANG
Subject: RE: about FDA position

Hi Dr. Wang,

How are you? Yes, I missed the conference this time. Are you attending ASP?

Yes, there is a possibility for creation of a position for a mass spec person. Can you please tell me what

you have a green card or citizenship? If you are looking for a permanent position you need citizenship and the position are difficult to get but there is always a chance.
Please understand that this is very preliminary talk, I will talk with my supervisors and director and let you know further.
Best regards
Rahul

From: YANHONG WANG [mailto:wangyh@olemiss.edu]
Sent: Friday, May 11, 2012 11:04 AM
To: Pawar, Rahul
Subject: about FDA position

Dear Rahul,

I didn't see you on this year's FDA conference. How is your everything going?

Dr. Khan told me that FDA had an open position for instrumental analysis on natural products, and suggested me to have your help. Would you please provide me more information on this position? If I want to apply for it, how should I prepare for it? Thank you very much.

Best regards,

Yan-Hong Wang

From: Oxford ICSB
To: [Pawar, Rahul](#)
Subject: Re: Abstract Submission
Date: Friday, March 19, 2010 10:02:32 AM

Dear Rahul,

The ICSB organizing committee would like to thank you for your contribution to the up-coming conference. This email is a confirmation that your abstracts have been selected for a poster presentations. The ICSB poster session will be held on the evening of Tuesday, April 13th, 2010. The maximum poster size is 42 X 42 inches. The poster boards will be available during the entire conference for presentation. We look forward to seeing you at this event. Best regards.

International Conference on the Science of Botanicals
National Center for Natural Products Research
University MS 38677
Phone 662-915-1090
Fax 662-915-7989
Email ICSB@olemiss.edu
www.OxfordICSB.org

From: "Pawar, Rahul" <Rahul.Pawar@fda.hhs.gov>
Date: Fri, 11 Dec 2009 15:22:54 -0500
To: <ICSB@olemiss.edu>
Conversation: Abstract Submission
Subject: Abstract Submission

Dear Troy,
Please find attached my two abstracts for the ICSB meeting.
I hope all is going well at your end, See you
Best regards
Rahul

<<ICSB10-Acacia-Abstract.doc>> <<ICSB10-PA-ChiralHPLC-Abstract.doc>>

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 301-436-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: ["bavula@olemiss.edu"](mailto:bavula@olemiss.edu)
Subject: RE: Acacia Information
Date: Monday, January 11, 2010 3:44:39 PM

Thank you Bharatiji
Talk to you soon

From: bavula@olemiss.edu [mailto:bavula@olemiss.edu]
Sent: Monday, January 11, 2010 12:42 PM
To: ""Pawar; Pawar, Rahul
Subject: RE: Acacia Information

Please find enclosed the Methodology for Acacia.

Thank you
Bharathi

On Friday 01/08/2010 at 2:30 pm, "Pawar, Rahul" wrote:

Hello Mam,
When you find time can you please send me the extraction method you used for the acacia samples.
Have a good weekend.
Rahul

-----Original Message-----

From: bavula@olemiss.edu [mailto:bavula@olemiss.edu]
Sent: Friday, October 23, 2009 10:20 AM
To: Pawar, Rahul
Subject: Acacia Information

Please find enclosed the files for Acacia. I have also enclosed the Phytochemistry paper.

Today we are having the poster session at NCNPR (busy day).

have a good weekend.
Bharathi

From: [Pawar, Rahul](#)
To: [BHARATHI AVULA](#)
Subject: RE: acacia manuscript
Date: Thursday, April 10, 2014 10:23:00 AM

No, we did not test for that.

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Thursday, April 10, 2014 10:10 AM
To: Pawar, Rahul
Subject: RE: acacia manuscript

Thank you Rahul...

In your paper I do not see you found methyl synephrine

bharathi

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Thursday, April 10, 2014 9:02 AM
To: BHARATHI AVULA
Subject: RE: acacia manuscript

Here you go..

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Thursday, April 10, 2014 9:57 AM
To: Pawar, Rahul
Subject: acacia manuscript

Could you please email me your acacia published manuscript

Thank you
bharathi

From: [Pawar, Rahul *](#)
To: ["BHARATHI AVULA"](#)
Subject: RE: Acacia
Date: Friday, September 16, 2011 10:36:08 AM

No Problem, thank you. You all have a good weekend. We are going to get very cold this weekend.

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Thursday, September 15, 2011 5:33 PM
To: Pawar, Rahul *
Subject: RE: Acacia

These two files only I could find from my computer please..

Thank you
Bharathi

From: Pawar, Rahul * [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Thursday, September 15, 2011 2:15 PM
To: BHARATHI AVULA
Subject: RE: Acacia

Thanks you!
These were the file you send me last time but I feel there is one more file that had information on the samples you analyzed. I feel it had some tables in it...

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Thursday, September 15, 2011 2:27 PM
To: Pawar, Rahul *
Subject: RE: Acacia

It is long time over and I am not sure if this is the right one or not....

Please find enclosed the files...

Parents are fine and will be going to India on 27th Oct 2011...

Have a good day...
Bharathi

From: Pawar, Rahul * [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Thursday, September 15, 2011 9:26 AM
To: BHARATHI AVULA
Subject: Acacia

Hello Bhatathiji,
How are you? When are uncle-aunty going back to india? Convery our regards to them.
Can you please resend me the Acacia word file you send me on 10/23/09. It had the data and method

you analyzed the acacia plant materials. Some how the file is not opening as our outlooks got converted from XP to windows 7.

Thank you

Rahul

Rahul Pawar Ph.D.

Division of Bioanalytical Chemistry

CFSAN-FDA

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 240-402-1795

Fax: 301-436-2622

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Re: Acacia
Date: Wednesday, June 20, 2012 3:08:04 PM

Great, Thanks. I am sure you feel good shipping it since you do not have to work on it.

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>

Date: Wed, 20 Jun 2012 15:06:33 -0400

To: Ikhlas Khan <ikhlan@olemiss.edu>

Subject: RE: Acacia

Sir,

I have shipped (Fedex) approx 2.7 kg of Twigs and 1.5 kg of leaf over dry ice. You should have it tomorrow.

Rahul

From: Ikhlas Khan[<mailto:ikhlan@olemiss.edu>]

Sent: Wednesday, June 20, 2012 2:47 PM

To: Pawar, Rahul

Subject: Re: Acacia

Thanks, that will be great.

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>

Date: Wed, 20 Jun 2012 12:28:55 -0400

To: Ikhlas Khan <ikhlan@olemiss.edu>

Subject: RE: Acacia

Dear Dr. Khan,

How are you?

I can send 2.5 kg of Acacia twigs withleaves and 1.5 kg of separated leaves. All material is at -78 and will be shipped on dry ice today or tomorrow.

Let me know if that will be enough foryou?

Thank you

Rahul

From: Ikhlas Khan[<mailto:ikhlan@olemiss.edu>]

Sent: Tuesday, May 22, 2012 12:18 PM

To: Pawar, Rahul

Subject: Re: Acacia

I am out till 31st. There is no rush from our side, I will get in touch once I get back.

IK

From: "Pawar, Rahul" <Rahul.Pawar@fda.hhs.gov>

Date: Tue, 22 May 2012 08:59:06 -0400

To: Ikhlas Khan <ikh@olemiss.edu>

Subject: Acacia

Good morning Dr. Khan

Will you be available to talk with Jeanne and myself around 11.30 am today?

Thank you

Rahul

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Re: Acaia rigidula
Date: Tuesday, April 7, 2015 12:34:48 PM

Yes, You are getting publicity but overall bad press for FDA
ik

From: <Pawar>, Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tuesday, April 7, 2015 at 9:29 AM
To: Ikhlas Khan <ikh@olemiss.edu>
Subject: Acaia rigidula

FYI- Acacia in news again.

<https://www.pharmamedtechbi.com/publications/the-tan-sheet/23/15/bmpea-in-supplements-spells-trouble-to-researchers>
<http://www.reuters.com/article/2015/04/07/health-dietarysupplements-stimulant-idUSL2N0X400U20150407>

Rahul

Rahul Pawar, Ph.D.
Office of Regulatory Science
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-3077

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE: Acaia rigidula
Date: Tuesday, April 7, 2015 12:46:00 PM

We never get any publicity but definably this is good for Dr. Cohen!

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Tuesday, April 07, 2015 12:35 PM
To: Pawar, Rahul
Subject: Re: Acaia rigidula

Yes, You are getting publicity but overall bad press for FDA
ik

From: <Pawar>, Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tuesday, April 7, 2015 at 9:29 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Acaia rigidula

FYI- Acacia in news again.

<https://www.pharmamedtechbi.com/publications/the-tan-sheet/23/15/bmpea-in-supplements-spells-trouble-to-researchers>
<http://www.reuters.com/article/2015/04/07/health-dietarysupplements-stimulant-idUSL2N0X400U20150407>

Rahul

Rahul Pawar, Ph.D.
Office of Regulatory Science
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-3077

From: Pawar, Rahul
To: Ikhlas Khan
Subject: RE: Acaia rigidula
Date: Friday, April 24, 2015 10:31:00 AM

Thank you!

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Friday, April 24, 2015 10:00 AM
To: Pawar, Rahul
Subject: Re: Acaia rigidula

Yes, I do. Good contribution, you should be recognized in FDA for your effort
ik

From: <Pawar>, Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Friday, April 24, 2015 at 8:15 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Acaia rigidula

Good morning, I hope you know by now that BMPEA was banned by FDA
<http://www.fda.gov/Food/DietarySupplements/QADietarySupplements/ucm443790.htm>

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Tuesday, April 07, 2015 12:35 PM
To: Pawar, Rahul
Subject: Re: Acaia rigidula

Yes, You are getting publicity but overall bad press for FDA
ik

From: <Pawar>, Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tuesday, April 7, 2015 at 9:29 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Acaia rigidula

FYI- Acacia in news again.

<https://www.pharmamedtechbi.com/publications/the-tan-sheet/23/15/bmpea-in-supplements-spells-trouble-to-researchers>
<http://www.reuters.com/article/2015/04/07/health-dietarysupplements-stimulant-idUSL2N0X400U20150407>

Rahul

Rahul Pawar, Ph.D.
Office of Regulatory Science

CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-3077

From: BHARATHI AVULA
To: [Pawar, Rahul](mailto:Pawar.Rahul)
Subject: RE: access to USP
Date: Friday, March 27, 2015 10:33:08 AM

Thank U very much

bharathi

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Friday, March 27, 2015 9:28 AM
To: BHARATHI AVULA
Subject: RE: access to USP

<http://www.uspnf.com/uspnf/pub/index?usp=37&nf=32&s=2&officialOn=December 1, 2014>

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Friday, March 27, 2015 10:22 AM
To: Pawar, Rahul
Subject: RE: access to USP

I do not have access to call U and also I am in basement, my cell does not work

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Friday, March 27, 2015 9:16 AM
To: BHARATHI AVULA
Subject: RE: access to USP

Is this what you want? If not call me, we can find it together.

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Friday, March 27, 2015 9:33 AM
To: Pawar, Rahul
Subject: RE: access to USP

Could U please check Second Supplement to USP 37-NF 32 on December 1, 2014, if limit test of Rutin (NMT 4 %) and Quercetin, (NMT 0.4%) for Ginkgo has been listed.
Also the specifications K/Q should be greater than 0.7

If possible could U please make a pdf and mail me (also site address).

Thanks
bharathi

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Friday, March 27, 2015 8:29 AM

To: BHARATHI AVULA

Subject: RE: access to USP

I think yes, let me know what.

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]

Sent: Friday, March 27, 2015 9:23 AM

To: Pawar, Rahul

Subject: access to USP

Do U have access to USP online?...if so I need you to check for some information

Thanks

bharathi

From: TROY J SMILLIE
To: [Pawar, Rahul](#)
Subject: Re: Address
Date: Tuesday, September 6, 2011 2:44:05 PM

Dear Rahul,

It is Pinus cembra and we have 8 grams. This is a voucher sample that came from Missouri Botanical Gardens.

Let me know if you are interested in getting this sample as well. The others are ready to ship (what quantities we had).

Troy Smillie, Ph.D.
Senior Research Scientist
Thad Cochran National Center for Natural Products Research
School of Pharmacy
University of Mississippi
University, MS 38677
Phone 662-915-1168
Fax 662-915-7062
<http://www.olemiss.edu/depts/pharmacy/php/sopquery3.php?id=69>

From: Pawar Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tue, 6 Sep 2011 10:20:32 -0400
To: Troy Smillie <tsmillie@olemiss.edu>
Subject: RE: Address

Troy,
Can you please provide more information about the nuts you have for us- type of nuts and the quantity and are they authenticated?
Thank you
Rahul

From: TROY J SMILLIE [mailto:tsmillie@olemiss.edu]
Sent: Friday, September 02, 2011 4:41 PM
To: Pawar, Rahul *
Subject: Re: Address

Thanks Rahul,

Vijay is getting the samples together. On a related note Jean had asked us for a sample of Pine nut. We have acquired an authentic sample but it is in small quantities. Does she still need it?

Troy Smillie, Ph.D.
Senior Research Scientist
Thad Cochran National Center for Natural Products Research
School of Pharmacy
University of Mississippi

University, MS 38677
Phone 662-915-1168
Fax 662-915-7062
<http://www.olemiss.edu/depts/pharmacy/php/sopquery3.php?id=69>

From: Pawar Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Fri, 2 Sep 2011 15:09:28 -0400
To: Troy Smillie <tsmillie@olemiss.edu>
Subject: Address

Hi Troy,
My address is

Rahul Pawar
Division of Bioanalytical Chemistry
CFSAN/FDA
5100 Paint Branch Parkway (HFS-717)
College Park, MD, 20740.

Thank you and have a good weekend

Rahul

Rahul Pawar Ph.D.

CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: TROY J SMILLIE
To: [Pawar, Rahul](#)
Subject: Re: Address
Date: Tuesday, September 6, 2011 3:33:31 PM

Dear Rahul,

Here is the information for the pine nut collection. We are sending you the entire sample that we have. MOBOT has the voucher at their location and that is how it probably should be cited.

<http://www.tropicos.org/Specimen/100402142>

Troy Smillie, Ph.D.
Senior Research Scientist
Thad Cochran National Center for Natural Products Research
School of Pharmacy
University of Mississippi
University, MS 38677
Phone 662-915-1168
Fax 662-915-7062
<http://www.olemiss.edu/depts/pharmacy/php/sopquery3.php?id=69>

From: Pawar Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tue, 6 Sep 2011 14:53:28 -0400
To: Troy Smillie <tsmillie@olemiss.edu>
Subject: RE: Address

Dear Troy,
Please send the Pine nuts too. Also please send all the authentication documentation you have for the pine nuts (PDF file will be ok too).
Jeanne has specially thanked you for the nut sample.
Best regards
Rahul

From: TROY J SMILLIE [mailto:tsmillie@olemiss.edu]
Sent: Tuesday, September 06, 2011 2:44 PM
To: Pawar, Rahul *
Subject: Re: Address

Dear Rahul,

It is Pinus cembra and we have 8 grams. This is a voucher sample that came from Missouri Botanical Gardens.

Let me know if you are interested in getting this sample as well. The others are ready to ship (what quantities we had).

Troy Smillie, Ph.D.
Senior Research Scientist
Thad Cochran National Center for Natural Products Research

School of Pharmacy
University of Mississippi
University, MS 38677
Phone 662-915-1168
Fax 662-915-7062
<http://www.olemiss.edu/depts/pharmacy/php/sopquery3.php?id=69>

From: Pawar Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tue, 6 Sep 2011 10:20:32 -0400
To: Troy Smillie <tsmillie@olemiss.edu>
Subject: RE: Address

Troy,
Can you please provide more information about the nuts you have for us- type of nuts and the quantity and are they authenticated?
Thank you
Rahul

From: TROY J SMILLIE [mailto:tsmillie@olemiss.edu]
Sent: Friday, September 02, 2011 4:41 PM
To: Pawar, Rahul *
Subject: Re: Address

Thanks Rahul,

Vijay is getting the samples together. On a related note Jean had asked us for a sample of Pine nut. We have acquired an authentic sample but it is in small quantities. Does she still need it?

Troy Smillie, Ph.D.
Senior Research Scientist
Thad Cochran National Center for Natural Products Research
School of Pharmacy
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Date: Fri, 2 Sep 2011 15:09:28 -0400
To: Troy Smillie <tsmillie@olemiss.edu>
Subject: Address

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My address is

Rahul Pawar
Division of Bioanalytical Chemistry
CFSAN/FDA
5100 Paint Branch Parkway (HFS-717)
College Park, MD, 20740.

Thank you and have a good weekend

Rahul

Rahul Pawar Ph.D.

CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: [Ikhlas Khan](#)
Subject: RE: Announcement
Date: Friday, September 9, 2016 8:26:00 AM

Congratulations Sir! Didn't come as a surprise the best choice.

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Thursday, September 08, 2016 4:15 PM
To: Pawar, Rahul
Subject: FW: Announcement

fyi

From: "sopdean@olemiss.edu" <sopdean@olemiss.edu>
Date: Thursday, September 8, 2016 at 2:15 PM
Subject: Announcement

To: Faculty, Research Scientists and Staff
From: David D. Allen, R.Ph., Ph.D.
Dean and Professor

School of Pharmacy Family,

I am very pleased to announce that on the recommendation of the search committee, I have extended an offer to Dr. Ikhlas Khan which he has accepted for the position of Director of the National Center for Natural Products Research (NCNPR) at the University of Mississippi School of Pharmacy. His position will become effective on January 1, 2017.

Ikhlas is an Associate Director at the NCNPR and Research Professor in the Division of Pharmacognosy, Department of BioMolecular Sciences. He brings a wealth of knowledge and experience to this position and we are excited that he is joining us in this capacity.

I'd like to thank the search committee and Chair, Dean Lee Cohen, for their stellar efforts in filling this position.

Please join me in congratulating Ikhlas on his new role with the School of Pharmacy!

David

David D. Allen, RPh, PhD, FASHP, FNAP, FAPhA
Dean and Professor
Executive Director of the Research Institute of Pharmaceutical Sciences
The University of Mississippi School of Pharmacy
P.O. Box 1848
University, MS 38677-1848

(662) 915-7265

Email: allen@olemiss.edu

From: [Pawar, Rahul](#)
To: [SHABANA I KHAN](#)
Subject: RE: anticancer testing
Date: Thursday, February 27, 2014 12:28:00 PM

Thank you Mam!

From: SHABANA I KHAN [<mailto:skhan@olemiss.edu>]
Sent: Thursday, February 27, 2014 12:12 PM
To: Pawar, Rahul
Subject: Re: anticancer testing

Please see attached papers. Since there is no activity you can add a few sentences and give the reference for method of assay. You don't need to include me in the paper. Acknowledgement will be enough.

Shabana Khan, Ph.D.
Principal Scientist
Room 2035 NCNPR, School of Pharmacy
University of Mississippi MS 38677
Phone: 662-9151041

From: <Pawar>, Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Wednesday, February 26, 2014 3:58 PM
To: Shabana Khan <skhan@olemiss.edu>
Subject: RE: anticancer testing

Dear Dr. Shabana,

Thank you for the results. Please include the compounds in any other assays you think will be appropriate.

Can you please also send me a write up of the materials and methods? If you are busy send me your published paper that has the same procedure and I will send a draft for you to correct. Thanks again for your timely help.

Best regards

Rahul

From: SHABANA I KHAN [<mailto:skhan@olemiss.edu>]
Sent: Wednesday, February 26, 2014 3:48 PM
To: Pawar, Rahul
Subject: anticancer testing

Dear Rahul,

Your samples did not show any anti-cell proliferative activity towards the four cancer cell lines and two noncancer cell lines up to a highest tested concentration of 25 µg/mL.

Shabana

Shabana Khan, Ph.D.

Principal Scientist

Room 2035 NCNPR, School of Pharmacy

University of Mississippi MS 38677

Phone: 662-9151041

From: SATYANARAYANARAJU SAGI
To: [Pawar, Rahul](#)
Subject: RE: Application for research position in your group
Date: Friday, August 21, 2015 2:49:57 PM

Thank you for your reply. It will be really great opportunity for me to work in your team in FDA.

Will keep in touch with you

Thank you
Sagi

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Friday, August 21, 2015 1:43 PM
To: SATYANARAYANARAJU SAGI
Cc: Ikhlas Khan
Subject: RE: Application for research position in your group

Hi Sagi,
Thanks for your interest. We don't have the position available currently but we are working on it. I will keep you posted if this materializes, hopefully by the end of this year.
Best wishes,
Rahul

From: SATYANARAYANARAJU SAGI [mailto:ssagi@olemiss.edu]
Sent: Friday, August 21, 2015 2:22 PM
To: Pawar, Rahul
Cc: Ikhlas Khan
Subject: Application for research position in your group

Dear Dr. Rahul,

I Satyanarayanaraju Sagi, introduce myself as a Post Doctoral Research Associate, working with Dr. Ikhlas Khan, Associate Director, NCNPR, University of Mississippi, Univeristy, Mississippi, USA. During my post doctoral research program, I have been working in analytical department which mainly focus on development of chemical fingerprint profiles of plant extracts using HPTLC, HPLC, UHPLC and UHPLC-MS/MS, the major applicability of these methods are in analyzing dietary supplements claiming to specific plant extracts.

My doctoral work was carried under the guidance of Dr. R. Nageswara Rao, Chief Scientist in Analytical chemistry division. My doctoral thesis mainly focused on bioanalytical method development and validation of anticancer and antihypersensitive drugs. The methods include novel blood collection technique like dried blood spots and ionic liquid based liquid-liquid microextraction from rat serum. My research work also include method of reverse phase chiral LC-MS for chiral separation and in-vitro disposition of chiral enantiomers. During this period, as an active member in natural product group my contribution involves method development for preparative isolation of

bioactive from the plant extracts and development fingerprinting profile, quantification of marker compounds in various medicinal plants. I will be helping the research scientist in structure elucidation of new isolates through fragmentation studies on mass spectrometry and conformation by high resolution mass spectrometric studies.

Having more than eight years of experience in the field of analytical chemistry with various expertise's I am keen in exploring an opportunity to work in the area of natural products and dietary supplements, which unambiguously would provide me an opportunity to further improve my knowledge and skills. Considering the interest to reach new heights in the field of analytical chemistry/dietary supplements and with all confidence that I shall be a potential competitor for Research Position in your research group, herewith I am enclosing my curriculum vitae for your kind perusal.

Hope my experience and skills meet your requirements. I feel privileged to furnish more information if you need.

Thank you for your consideration and I hope to hear from you in the near future.

Sagi

Satyanarayanaraju Sagi
Post Doctoral Research Associate
National Center for Natural Products Research
School of Pharmacy
The University of Mississippi
University, MS 38677
Work: 662 915 7610
Email: ssagi@olemiss.edu; rajussnpharma132@gmail.com

From: [Pawar, Rahul](#)
To: [SATYANARAYANARAJU SAGI](#)
Subject: RE: Application for research position in your group
Date: Tuesday, May 24, 2016 12:16:00 PM

Ok. ORISE people will contact you to make travel arrangements. Thanks.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Tuesday, May 24, 2016 12:12 PM
To: Pawar, Rahul
Subject: RE: Application for research position in your group

Yes, 22nd June looks fine me.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Tuesday, May 24, 2016 11:02 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Application for research position in your group

22 June work for you? You will have to travel to DC on 21st.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Monday, May 23, 2016 4:25 PM
To: Pawar, Rahul
Subject: RE: Application for research position in your group

No Problem

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Monday, May 23, 2016 3:21 PM
To: SATYANARAYANARAJU SAGI
Subject: RE: Application for research position in your group

Raju,
Please disregard the forms I send you today. We are planning to have funding for your travel through different source.
Sorry for the trouble,
Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Monday, May 23, 2016 11:44 AM
To: Pawar, Rahul

Subject: RE: Application for research position in your group

Dear Rahul,

No, I didn't receive any messages from you. Is it onsite talk or phone talk?

My contact no (b) (6)

Thanks

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Monday, May 23, 2016 10:38 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Application for research position in your group

Hi Raju,

Do you get my phone messages last Friday? Let me know when you are available to talk.

Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Monday, April 04, 2016 11:58 AM
To: Pawar, Rahul
Subject: RE: Application for research position in your group

Dear Rahul,

Yes, sure I will available to discuss with anytime during the conference.

See you soon

Thanks

Sagi

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Monday, April 04, 2016 10:47 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Application for research position in your group

Hi Sagi,

Are you attending the conference next week? I wanted to talk with you if you are in oxford during that time.

Best wishes,

Rahul

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: RE: Are you all coming for ASP?????.....bharathi
Date: Thursday, July 31, 2014 11:07:13 AM

OK...we will miss you

Good luck
bharathi

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Thursday, July 31, 2014 9:18 AM
To: BHARATHI AVULA
Subject: RE: Are you all coming for ASP?????.....bharathi

Hi Bharathi,
No I am not. I am attending ACS meeting next week where I have a talk. Thanks
Rahul

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Thursday, July 31, 2014 8:26 AM
To: Pawar, Rahul
Subject: Are you all coming for ASP?????.....bharathi

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: RE: ASP
Date: Thursday, July 26, 2012 1:04:43 PM

Yes please....we are coming to NY on Saturday.

Parents are doing fine and were asking about you all

Good luck

Bharathi

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Thursday, July 26, 2012 11:53 AM
To: BHARATHI AVULA
Subject: ASP

Hi Bharathi,
I hope you all are travelling to NY. How is Uncle and Aunty..
Convey our regards to both
Thanks
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: ["BHARATHI AVULA"](#)
Subject: RE: ASP
Date: Thursday, July 26, 2012 1:05:00 PM

See you!

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Thursday, July 26, 2012 1:05 PM
To: Pawar, Rahul
Subject: RE: ASP

Yes please....we are coming to NY on Saturday.

Parents are doing fine and were asking about you all

Good luck
Bharathi

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Thursday, July 26, 2012 11:53 AM
To: BHARATHI AVULA
Subject: ASP

Hi Bharathi,
I hope you all are travelling to NY. How is Uncle and Aunty..
Convey our regards to both
Thanks
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE: Bob Moore's Retirement Party -- October 27 -- Moose Creek Steakhouse, College Park
Date: Tuesday, October 18, 2011 3:38:52 PM

Nothing, as far as I know. Anything specific?

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Tuesday, October 18, 2011 2:21 PM
To: Pawar, Rahul
Subject: Re: Bob Moore's Retirement Party -- October 27 -- Moose Creek Steakhouse, College Park

Thanks, is there something going on.
IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tue, 18 Oct 2011 12:01:00 -0400
To: Ikhlas Khan <ikhan@olemiss.edu>
Subject: FW: Bob Moore's Retirement Party -- October 27 -- Moose Creek Steakhouse, College Park

FYI

From: CFSAN-ALLHANDS
Sent: Tuesday, October 18, 2011 11:12 AM
To: CFSAN-All Hands
Subject: Bob Moore's Retirement Party -- October 27 -- Moose Creek Steakhouse, College Park

After more than 23 distinguished years in Federal Service, Dr. Robert Moore, Mr. Dietary Supplement, has called it quits, and plans to return to the farm in South Dakota. Please join us for a fond farewell.

Location: Moose Creek Steakhouse (Holiday Inn) College Park, MD

Date: October 27, 2011

Time: 11:30 am – 2:30 pm

Cost: \$22.00 includes buffet/drink/tax/gratuuity and gift

Please RSVP by contacting Lisa Barr by email Lisa.Barr@fda.hhs.gov or phone (240)-402-2375, before October 25, 2011

(-OMS-)

Please note you are receiving this email because you are using an email account supported by the Department of Health and Human Services. Please do not respond to this email. If you have questions or comments regarding this specific message, please write to the responsible party. If you would like to submit a message for CFSAN All-Hands, please email "OMS Action".

From: VIJAYASANKAR RAMAN
To: [Pawar, Rahul](#)
Subject: RE: Botanicals
Date: Friday, September 2, 2011 4:19:41 PM

Got clearance from Dr. Troy !
I will try to ship the samples on Tuesday, after the holidays.
Have a good weekend...

Vijay

From: Pawar, Rahul * [Rahul.Pawar@fda.hhs.gov]
Sent: Friday, September 02, 2011 1:43 PM
To: VIJAYASANKAR RAMAN
Subject: RE: Botanicals

[Ok, thanks](#)

From: VIJAYASANKAR RAMAN [mailto:vraman@olemiss.edu]
Sent: Friday, September 02, 2011 2:42 PM
To: Pawar, Rahul *
Subject: RE: Botanicals

Dear Rahul

I have not received any information from Dr. Khan regarding sending of samples to you. And he is currently out of station.

Vijay.

From: Pawar, Rahul * [Rahul.Pawar@fda.hhs.gov]
Sent: Friday, September 02, 2011 1:36 PM
To: VIJAYASANKAR RAMAN
Subject: RE: Botanicals

[Hi Vijay,](#)
[Please let me know when you ship the material.](#)
[Thanks have a good weekend](#)
[Rahul](#)

From: VIJAYASANKAR RAMAN [mailto:vraman@olemiss.edu]
Sent: Tuesday, August 30, 2011 3:31 PM
To: Ikhlas Khan; Aruna Weerasooriya
Cc: Pawar, Rahul *
Subject: RE: Botanicals

Dear Rahul

We currently have authenticated samples of three species - *Acorus calamus*, *Piper methysticum* and *Tripterygium wilfordii*; and two commercial samples (not authenticated) viz. *Larrea tridentata* and *Teucrium chamaedrys*. If needed, I can help you with authentication of these samples.

Regards

Vijay

From: Ikhlas Khan
Sent: Tuesday, August 30, 2011 1:23 PM
To: Aruna Weerasooriya; VIJAYASANKAR RAMAN
Cc: Rahul
Subject: FW: Botanicals

Thanks Rahul

Aruna and Vijay will give you update on this. They have most of it but I am not sure which one.

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tue, 30 Aug 2011 13:05:59 -0400
To: Ikhlas Khan <ikhan@olemiss.edu>
Subject: RE: Botanicals

Hello Dr. Khan,
Happy ID to you all.
Any update about the plant materials?
Thank you
Rahul

From: Ikhlas Khan[<mailto:ikhan@olemiss.edu>]
Sent: Tuesday, August 09, 2011 3:04 PM
To: Pawar, Rahul
Subject: Re: Botanicals

It was our pleasure. We all enjoyed to be with your family. I will check with Vijay and Aruna and come back to you. I am sure we have most of them.

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tue, 9 Aug 2011 10:56:21 -0400
To: Ikhlas Khan <ikhan@olemiss.edu>
Subject: Botanicals

Hello Dr. Khan,

I hope you all are doing well. It was great to spend some wonderful time with you all at San Diego. I was in need of the following authenticated botanical materials (about 100 gm each), can you please provide them to us? If you are unable to provide some of these then I can buy this online (like Frontier Coop or Starwest) but then can any botanist on your lab help in the authentication?

1. Germander: *Teucrium chamaedrys*
2. Calamus root: *Acorus calamus*
3. Kava Kava: *Piper methysticum*
4. Chaparral: *Larrea tridentata*
5. Usnea: *Usnea* sp
6. Cascara sagrada bark
7. *Tripterygium wilfordii*

Please let me know if that is possible. Thank you.
Best regards,
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: VIJAYASANKAR RAMAN
To: [Pawar, Rahul](#)
Subject: RE: Botanicals
Date: Friday, September 2, 2011 2:43:07 PM

Dear Rahul

I have not received any information from Dr. Khan regarding sending of samples to you. And he is currently out of station.

Vijay.

From: Pawar, Rahul * [Rahul.Pawar@fda.hhs.gov]
Sent: Friday, September 02, 2011 1:36 PM
To: VIJAYASANKAR RAMAN
Subject: RE: Botanicals

Hi Vijay,
Please let me know when you ship the material.
Thanks have a good weekend
Rahul

From: VIJAYASANKAR RAMAN [mailto:vraman@olemiss.edu]
Sent: Tuesday, August 30, 2011 3:31 PM
To: Ikhlas Khan; Aruna Weerasooriya
Cc: Pawar, Rahul *
Subject: RE: Botanicals

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Regards

Vijay

From: Ikhlas Khan
Sent: Tuesday, August 30, 2011 1:23 PM
To: Aruna Weerasooriya; VIJAYASANKAR RAMAN
Cc: Rahul
Subject: FW: Botanicals

Thanks Rahul

Aruna and Vijay will give you update on this. They have most of it but I am not sure which one.

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tue, 30 Aug 2011 13:05:59 -0400
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Botanicals

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Happy ID to you all.
Any update about the plant materials?
Thank you
Rahul

From: Ikhlas Khan[<mailto:ikhlan@olemiss.edu>]
Sent: Tuesday, August 09, 2011 3:04 PM
To: Pawar, Rahul
Subject: Re: Botanicals

It was our pleasure. We all enjoyed to be with your family. I will check with Vijay and Aruna and come back to you. I am sure we have most of them.
IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tue, 9 Aug 2011 10:56:21 -0400
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Botanicals

Hello Dr. Khan,
I hope you all are doing well. It was great to spend some wonderful time with you all at San Diego. I was in need of the following authenticated botanical materials (about 100 gm each), can you please provide them to us? If you are unable to provide some of these then I can buy this online (like Frontier Coop or Starwest) but then can any botanist on your lab help in the authentication?

1. Germander: *Teucrium chamaedrys*
2. Calamus root: *Acorus calamus*
3. Kava Kava: *Piper methysticum*
4. Chaparral: *Larrea tridentata*
5. Usnea: *Usnea* sp
6. Cascara sagrada bark
7. *Tripterygium wilfordii*

Please let me know if that is possible. Thank you.
Best regards,
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE: Botanicals
Date: Tuesday, August 9, 2011 3:34:00 PM

Thank you Sir

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Tuesday, August 09, 2011 3:04 PM
To: Pawar, Rahul
Subject: Re: Botanicals

It was our pleasure. We all enjoyed to be with your family. I will check with Vijay and Aruna and come back to you. I am sure we have most of them.

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tue, 9 Aug 2011 10:56:21 -0400
To: Ikhlas Khan <ikhan@olemiss.edu>
Subject: Botanicals

Hello Dr. Khan,

I hope you all are doing well. It was great to spend some wonderful time with you all at San diego.

I was in need of the following authenticated botanical materials (about 100 gm each), can you please provide them to us? If you are unable to provide some of these then I can buy this online (like Frontier Coop or Starwest) but then can any botanist on your lab help in the authentication?

1. Germander: Teucrium chamaedrys
2. Calamus root: Acorus calamus
3. Kava Kava: Piper methysticum
4. Chaparral: Larrea tridentata
5. Usnea: Usnea sp
6. Cascara sagrada bark
7. Tripterygium wilfordii

Please let me know if that is possible. Thank you.

Best regards,
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul *](#)
To: ["VIJAYASANKAR RAMAN"](#)
Subject: RE: Botanicals
Date: Tuesday, September 6, 2011 9:20:00 AM

Hi Vijay,
My address is ..

Rahul Pawar
Division of Bioanalytical Chemistry
CFSAN/FDA
5100 Paint Branch Parkway (HFS-717)
College Park, MD, 20740.

Please let me know if the package has a tracking number when you ship.
Regards
Rahul

From: VIJAYASANKAR RAMAN [mailto:vraman@olemiss.edu]
Sent: Friday, September 02, 2011 4:20 PM
To: Pawar, Rahul *
Subject: RE: Botanicals

Got clearance from Dr. Troy !
I will try to ship the samples on Tuesday, after the holidays.
Have a good weekend...

Vijay

From: Pawar, Rahul * [Rahul.Pawar@fda.hhs.gov]
Sent: Friday, September 02, 2011 1:43 PM
To: VIJAYASANKAR RAMAN
Subject: RE: Botanicals

Ok, thanks

From: VIJAYASANKAR RAMAN [mailto:vraman@olemiss.edu]
Sent: Friday, September 02, 2011 2:42 PM
To: Pawar, Rahul *
Subject: RE: Botanicals

Dear Rahul

I have not received any information from Dr. Khan regarding sending of samples to you. And he is currently out of station.

Vijay.

From: Pawar, Rahul * [Rahul.Pawar@fda.hhs.gov]
Sent: Friday, September 02, 2011 1:36 PM
To: VIJAYASANKAR RAMAN
Subject: RE: Botanicals

Hi Vijay,

Please let me know when you ship the material.
Thanks have a good weekend
Rahul

From: VIJAYASANKAR RAMAN [mailto:vraman@olemiss.edu]
Sent: Tuesday, August 30, 2011 3:31 PM
To: Ikhlas Khan; Aruna Weerasooriya
Cc: Pawar, Rahul *
Subject: RE: Botanicals

Dear Rahul

We currently have authenticated samples of three species - *Acorus calamus*, *Piper methysticum* and *Tripterygium wilfordii*; and two commercial samples (not authenticated) viz. *Larrea tridentata* and *Teucrium chamaedrys*. If needed, I can help you with authentication of these samples.

Regards

Vijay

From: Ikhlas Khan
Sent: Tuesday, August 30, 2011 1:23 PM
To: Aruna Weerasooriya; VIJAYASANKAR RAMAN
Cc: Rahul
Subject: FW: Botanicals

Thanks Rahul

Aruna and Vijay will give you update on this. They have most of it but I am not sure which one.

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tue, 30 Aug 2011 13:05:59 -0400
To: Ikhlas Khan <ikhan@olemiss.edu>
Subject: RE: Botanicals

Hello Dr. Khan,
Happy ID to you all.
Any update about the plant materials?
Thank you
Rahul

From: Ikhlas Khan[<mailto:ikhan@olemiss.edu>]
Sent: Tuesday, August 09, 2011 3:04 PM
To: Pawar, Rahul
Subject: Re: Botanicals

It was our pleasure. We all enjoyed to be with your family. I will check with Vijay and Aruna and come back to you. I am sure we have most of them.

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>

Date: Tue, 9 Aug 2011 10:56:21 -0400

To: Ikhlas Khan <ikh@olemiss.edu>

Subject: Botanicals

Hello Dr. Khan,

I hope you all are doing well. It was great to spend some wonderful time with you all at San Diego.

I was in need of the following authenticated botanical materials (about 100 gm each), can you please provide them to us? If you are unable to provide some of these then I can buy this online (like Frontier Coop or Starwest) but then can any botanist on your lab help in the authentication?

1. Germander: *Teucrium chamaedrys*
2. Calamus root: *Acorus calamus*
3. Kava Kava: *Piper methysticum*
4. Chaparral: *Larrea tridentata*
5. Usnea: *Usnea* sp
6. Cascara sagrada bark
7. *Tripterygium wilfordii*

Please let me know if that is possible. Thank you.

Best regards,

Rahul

Rahul Pawar Ph.D.

Division of Bioanalytical Chemistry

CFSAN-FDA

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 240-402-1795

Fax: 301-436-2622

From: Ikhlas A. Khan
To: [Pawar, Rahul](#)
Subject: RE: CV
Date: Monday, June 28, 2010 4:39:18 PM

Hi Rahul

Anupam who is visiting us as part of CRISM exchange program would like to come and visit FDA and ofcourse see the Washington area. Jeanne said its Ok for you to spend few hrs with him and show him college park.

I am copying to him so you both can arrange it.

Thanks

IK

--

From: [Pawar, Rahul](#)
To: ["Jennifer Michael"](#)
Subject: RE: CV
Date: Tuesday, June 1, 2010 9:59:18 AM

Hi Jennifer,
This one is not the updated so I will use the one you provided me earlier.
Thank you for your help
Rahul

From: Jennifer Michael [mailto:jenmike@olemiss.edu]
Sent: Friday, May 28, 2010 2:24 PM
To: Pawar, Rahul
Subject: RE: CV

The only abbreviated one I have is 13 pages and I have it attached. Hope that will be okay.
Jennifer

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Friday, May 28, 2010 11:50 AM
To: Jennifer Michael
Subject: CV

Hi Jennifer,
Do you have a short CV for Dr Walker (5-10 pages). Can you please send me one?
Have a good weekend
Best regards
Rahul

From: [Pawar, Rahul](#)
To: ["Ikhlas A. Khan"](#)
Subject: RE: CV
Date: Tuesday, June 1, 2010 9:01:57 AM

Thank you Dr. Khan,
I am sure you can stretch it to even 100 pages long. But if you have little shorter CV (say 10-20) pages will be good enough for my application.
By the way when are you travelling to India?
I hope to see you at ASP.
Best regards
Rahul

From: Ikhlas A. Khan [mailto:ikhan@olemiss.edu]
Sent: Friday, May 28, 2010 4:30 PM
To: Pawar, Rahul
Subject: Re: CV

71 pages are not good enough, how long it should be. Attached, please find the newer version.
IK

Dear Dr Khan,
I hope you are doing well.
I received the recommendation letter, thank you very much for the timely help.
Can you also please send me your recent **short** CV, the one I have is 71 pages long!!
Thank you and have a good weekend
Rahul

--

From: [Pawar, Rahul](#)
To: ["Ikhlas A. Khan"](#)
Subject: RE: CV
Date: Monday, June 28, 2010 4:54:26 PM

Good to talk to you, you can give my phone to him- (b) (6)
Thank you for you help
Rahul

From: Ikhlas A. Khan [mailto:ikhan@olemiss.edu]
Sent: Monday, June 28, 2010 4:39 PM
To: Pawar, Rahul
Subject: RE: CV

Hi Rahul

Anupam who is visiting us as part of CRISM exchange program would like to come and visit FDA and ofcourse see the Washington area. Jeanne said its Ok for you to spend few hrs with him and show him college park.

I am copying to him so you both can arrange it.

Thanks

IK

--

From: [Pawar, Rahul](#)
To: [BHARATHI AVULA](#)
Subject: RE: Dietary supplements labeled as blood sugar management (purchased)
Date: Thursday, August 27, 2015 2:52:00 PM

Ok, thanks!

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Thursday, August 27, 2015 1:58 PM
To: Pawar, Rahul
Cc: Ikhlas Khan
Subject: RE: Dietary supplements labeled as blood sugar management (purchased)

Thank you Rahul for the list

After Dr. Khan is back from China I will discuss with him

bharathi

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Thursday, August 27, 2015 10:28 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>; BHARATHI AVULA <bavula@olemiss.edu>
Subject: Dietary supplements labeled as blood sugar management (purchased)

Hello!

Here is the list of the sugar management products we purchased. These were purely chosen based on the label claim and not by ingredient. So, for example, we have not collected supplements just selling Moringa extract, unless it made a health related claim.

Let me know if you can analyze these products for their botanical ingredient? Thanks.

Best wishes,

Rahul

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Re: Duke database
Date: Monday, October 26, 2015 12:04:55 PM

Yes, that will be good. Visit him and ask what we need to have to maintain it. He knows me and I sent him an email

ik

From: Rahul Pawar <Rahul.Pawar@fda.hhs.gov>
Date: Monday, October 26, 2015 at 10:58 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Duke database

Will be a good idea to communicate with Dr. Duke. He lives nearby, we can visit him if possible. See you..

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Monday, October 26, 2015 11:48 AM
To: Pawar, Rahul
Subject: Re: Duke database

Thursday

ik

From: Rahul Pawar <Rahul.Pawar@fda.hhs.gov>
Date: Monday, October 26, 2015 at 10:47 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Duke database

Ok, when are you reaching?

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Monday, October 26, 2015 11:40 AM
To: Pawar, Rahul
Subject: Re: Duke database

Yes, I am interested in it but I will check what it needs to maintain. I will be talking about it once I reach there

ik

From: Rahul Pawar <Rahul.Pawar@fda.hhs.gov>
Date: Monday, October 26, 2015 at 10:28 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Duke database

Hello Dr. Khan,

Just came to know something about James Duke's NP database which I thought you might be interested in. USDA will not be hosting and updating the database by this yearend and Dr. Duke is offering it to anyone who might be interested in hosting it. Let me know if UM is interested in having it, I will provide more information as I get. TTY soon.

Best wishes,

Rahul

Rahul Pawar, Ph.D.

Office of Regulatory Science

CFSAN-FDA

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 240-402-1795

Fax: 301-436-3077

From: bavula@olemiss.edu
To: [Pawar, Rahul](#)
Subject: RE: EB1
Date: Wednesday, January 12, 2011 8:38:39 AM

University is opened today and roads are very bad....

Thank you for your help

Bharathi

On Tuesday 01/11/2011 at 10:38 am, "Pawar, Rahul" wrote:

I heard that but not aware that the university is closed.
Since your (EB2) I-485 is already filed you have to only file I-140 form again, it is the same form for EB2 also, you just change the category to "researcher with extraordinary ability". You have to file only one form along with your supporting documents like petition letter, reco letters publication prints etc...
Will talk in details
Rahul

From: bavula@olemiss.edu [mailto:bavula@olemiss.edu]
Sent: Tuesday, January 11, 2011 9:46 AM
To: Pawar, Rahul
Subject: RE: EB1

We had a severe winter storm and University is closed. Today also the University is closed....may be tomorrow will be opened but not sure....

Last sunday I called you but no response....

Thank you for email id of Jeanne...

Bharathi

On Monday 01/10/2011 at 1:02 pm, "Pawar, Rahul" wrote:

I called several times today, call me when you are available
Her id is Jeanne.Rader@fda.hhs.gov

From: bavula@olemiss.edu [mailto:bavula@olemiss.edu]
Sent: Friday, January 07, 2011 5:17 PM
To: Pawar, Rahul
Subject: EB1

Could you please mail me the EB1 form and also one-two letters...

I am getting lost...

I should be able to take letters from your boss (i need her email id)
and others...

Thank you
Bharathi

From: [Pawar, Rahul](#)
To: ["bavula@olemiss.edu"](mailto:bavula@olemiss.edu)
Subject: RE: Emailing: jaoi.89.4
Date: Thursday, October 7, 2010 11:38:00 AM

I don't have internet at my home.

You should start to write reco letters, they are not different then one written for EB2, just little stronger and focused. I actually modified my eb2 letters this time. Decide form whom you want the letters this time.

From: bavula@olemiss.edu [mailto:bavula@olemiss.edu]
Sent: Thursday, October 07, 2010 11:34 AM
To: Pawar, Rahul
Subject: Re: Emailing: jaoi.89.4

Thank you

No please...You mailed me only your letter and waiting for others.

I need to read the mails and then decide.

Thank you
Bharathi

On Thursday 10/07/2010 at 10:29 am, "Pawar, Rahul" wrote:
Have you started with you Eb1?

From: [Pawar, Rahul](#)
To: ["bavula@olemiss.edu"](mailto:bavula@olemiss.edu)
Subject: RE: Emailing: jaoi.89.4
Date: Thursday, October 7, 2010 12:15:00 PM

Yes I will
Regards to uncle and aunty

From: bavula@olemiss.edu [mailto:bavula@olemiss.edu]
Sent: Thursday, October 07, 2010 11:41 AM
To: Pawar, Rahul
Subject: RE: Emailing: jaoi.89.4

No problems...

I will try to write one letter and mail you. Check for me if it is ok...

if possible mail me your house photos...Parents are happy about you both settling and would like to see child and house photos.

Thank you

Bharathi

On Thursday 10/07/2010 at 10:38 am, "Pawar, Rahul" wrote:

I don't have internet at my home.

You should start to write reco letters, they are not different then one written for EB2, just little stronger and focused. I actually modified my eb2 letters this time. Decide form whom you want the letters this time.

From: bavula@olemiss.edu [mailto:bavula@olemiss.edu]
Sent: Thursday, October 07, 2010 11:34 AM
To: Pawar, Rahul
Subject: Re: Emailing: jaoi.89.4

Thank you

No please...You mailed me only your letter and waiting for others.

I need to read the mails and then decide.

Thank you

Bharathi

On Thursday 10/07/2010 at 10:29 am, "Pawar, Rahul" wrote:

Have you started with you Eb1?

From: bavula@olemiss.edu
To: [Pawar, Rahul](#)
Subject: Re: Emailing: jaoi.90.3
Date: Wednesday, July 21, 2010 2:25:46 PM

Thank you very much...

Congrats Rahul....Bahut Bada Treat....

Bharathi

On Wednesday 07/21/2010 at 1:23 pm, "Pawar, Rahul" wrote:

<<jaoi.90.3.pdf>>
Here are your papers
Sorry for the delay

From: bavula@olemiss.edu
To: [Pawar, Rahul](#)
Subject: Re: Emailing: jaoi.90.5
Date: Friday, June 18, 2010 9:03:22 AM

Thank you Rahul.

Valli is again admitted in the hospital as she has clot in the left leg. Now she seems to be fine.

Have a nice day and good weekend...

Bharathi

On Friday 06/18/2010 at 7:56 am, "Pawar, Rahul" wrote:

<<jaoi.90.5.pdf>>

From: [Pawar, Rahul](#)
To: ["bavula@olemiss.edu"](mailto:bavula@olemiss.edu)
Subject: RE: Emailing: jaoi.91.6
Date: Friday, September 24, 2010 2:29:00 PM

You too!

From: bavula@olemiss.edu [mailto:bavula@olemiss.edu]
Sent: Friday, September 24, 2010 2:29 PM
To: Pawar, Rahul
Subject: Re: Emailing: jaoi.91.6

Thank you

have a good weekend

Bharathi

On Friday 09/24/2010 at 1:28 pm, "Pawar, Rahul" wrote:

From: Kathy Strawn
To: [Pawar, Rahul](#)
Subject: RE: FED EX
Date: Wednesday, May 12, 2010 3:25:37 PM

Hope they help

Kathy Strawn
Senior Administrative Secretary
National Center for Natural Products Research
P.O. Box 1848
University, MS 38677
Phone (662) 915-1090
Fax (662) 915-7989
Email mstrawn@olemiss.edu

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Wednesday, May 12, 2010 2:25 PM
To: mstrawn@olemiss.edu
Subject: RE: FED EX

Yes, I just got it.
Thank you very much for your timely help
Best regards
Rahul

From: Kathy Strawn [mailto:mstrawn@olemiss.edu]
Sent: Wednesday, May 12, 2010 3:23 PM
To: Pawar, Rahul
Subject: FED EX

Did you get the Fed Ex'ed letters today?

Kathy Strawn
Senior Administrative Secretary
National Center for Natural Products Research
P.O. Box 1848
University, MS 38677
Phone (662) 915-1090
Fax (662) 915-7989
Email mstrawn@olemiss.edu

From: bavula@olemiss.edu
To: Pawar, Rahul
Subject: RE: Format of publications in CV
Date: Tuesday, January 18, 2011 11:15:06 AM

Thank you very much

Bharathi

On Tuesday 01/18/2011 at 10:13 am, "Pawar, Rahul" wrote:

This is from my Cv, Follow this one

8. Peer-reviewed Publications

1. Pawar, R. S., Gopalakrishnan, C., and Bhutani, K. K. 2001. Dammarane triterpene saponin from *Bacopa monniera* as the superoxide inhibitor in polymorphonuclear cells. *Planta Medica*, 67: 752-754.
2. Pawar, R. S. and Bhutani, K. K. 2004. Madhucosides A and B, protobassic acid glycosides from *Madhuca indica* with inhibitory activity on free radical release from phagocytes. *Journal of Natural Products*, 67: 668-671.
3. Ganzera, M., Gampenrieder, J., Pawar, R. S., Khan, I. A., and Stuppner, H. 2004. Separation of the major triterpenoid saponins in *Bacopa monniera* by high-performance liquid chromatography. *Analytica Chimica Acta*, 516: 149-154.

From: bavula@olemiss.edu [mailto:bavula@olemiss.edu]
Sent: Tuesday, January 18, 2011 9:09 AM
To: Pawar, Rahul
Subject: Format of publications in CV

Could you please email me 1-2 publications format to be presented in CV (is there is any specific format)....

Thank you
Bharathi

From: [Rader, Jeanne I](#)
To: [Pawar, Rahul](#)
Subject: RE: From Ikhlas - last week
Date: Monday, May 2, 2011 2:15:48 PM

Thanks - you probably know where he lived and whether other colleagues lived near the family.

Jeanne I. Rader, Ph.D.
Director, Division of Bioanalytical Chemistry
Office of Regulatory Science
CFSAN, Food and Drug Admin.
5100 Paint Branch Parkway
College Park, MD 20740
Telephone: 301-436-1786
Fax: 301-436 2622
e-mail: Jeanne.Rader@fda.hhs.gov

-----Original Message-----

From: Pawar, Rahul
Sent: Monday, May 02, 2011 2:12 PM
To: Rader, Jeanne I
Subject: RE: From Ikhlas - last week

Very sad to hear this, I will call somebody (b) (6) today and get an update

-----Original Message-----

From: Rader, Jeanne I
Sent: Monday, May 02, 2011 2:08 PM
To: Pawar, Rahul; Tamta, Hemlata *
Subject: FW: From Ikhlas - last week
Importance: High

This is the sad news about Troy's house.

Jeanne I. Rader, Ph.D.
Director, Division of Bioanalytical Chemistry
Office of Regulatory Science
CFSAN, Food and Drug Admin.
5100 Paint Branch Parkway
College Park, MD 20740
Telephone: 301-436-1786
Fax: 301-436 2622
e-mail: Jeanne.Rader@fda.hhs.gov

-----Original Message-----

From: Ikhlas A. Khan [<mailto:ikhan@olemiss.edu>]
Sent: Wednesday, April 27, 2011 10:37 PM
To: Rader, Jeanne I
Subject:

we hear about the bad weather and this season has been very bad but we don't feel unless it happens to someone close to us. We had full two days of warning of tornados and storms. This afternoon tornado touched near (b) (6) and destroyed the neighborhood and Troy (b) (6) (b) (6) (b) (6). Luckily no one was at home.

IK

From: [Pawar, Rahul](#)
To: [BHARATHI AVULA](#)
Subject: RE: hai
Date: Friday, February 21, 2014 11:24:00 AM

Welcome back! I hope she feels better, Are they coming to US in summer?

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Friday, February 21, 2014 10:50 AM
To: Pawar, Rahul
Subject: hai

Dear Rahul and Hema,

I am back to work. Mom is slowly recovering

Thank you
bharathi

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: RE: hai
Date: Monday, May 16, 2011 8:42:41 AM

Dear Rahul,

I hope by this time you know what happened to Amar's house...

It is very bad and hope he will feel better with time....

Bharathi

From: [Pawar, Rahul](#)
To: ["bavula@olemiss.edu"](mailto:bavula@olemiss.edu)
Subject: RE: hai
Date: Monday, March 28, 2011 2:23:00 PM

Hi Bharathiji,
Yes, we will be there, soon. How about you, Did you file eb1?
TTY soon
Regards
Rahul

From: bavula@olemiss.edu [mailto:bavula@olemiss.edu]
Sent: Monday, March 28, 2011 2:19 PM
To: Pawar, Rahul
Subject: hai

hai dear Rahul,

how are you???

I hope this time also all of you are planning to visit oxford for FDA conference....

Best wishes
Bhaarthi

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: RE: hai
Date: Friday, February 21, 2014 11:44:11 AM

Not sure...depend how well she recovers...

bharathi

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Friday, February 21, 2014 10:25 AM
To: BHARATHI AVULA
Subject: RE: hai

Welcome back! I hope she feels better, Are they coming to US in summer?

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Friday, February 21, 2014 10:50 AM
To: Pawar, Rahul
Subject: hai

Dear Rahul and Hema,

I am back to work. Mom is slowly recovering

Thank you
bharathi

From: [Pawar, Rahul](#)
To: ["mstrawn@olemiss.edu"](mailto:mstrawn@olemiss.edu)
Subject: RE: Happy mothers day
Date: Tuesday, May 11, 2010 9:21:37 AM

Good morning Kathy,
Do you know, if Dr. Khan prepared and mailed my recommendation letter?
Please let me know
Thanks
Rahul

From: Kathy Strawn [mailto:mstrawn@olemiss.edu]
Sent: Monday, May 10, 2010 9:15 AM
To: Pawar, Rahul
Subject: RE: Happy mothers day

That's great. Tell (b) (6) hello for me.

Kathy Strawn
Senior Administrative Secretary
National Center for Natural Products Research
P.O. Box 1848
University, MS 38677
Phone (662) 915-1090
Fax (662) 915-7989
Email mstrawn@olemiss.edu

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Monday, May 10, 2010 8:10 AM
To: mstrawn@olemiss.edu
Subject: RE: Happy mothers day

I am Happy you had a great day.
(b) (6) had a good one too, with lots of gifts and a card from (b) (6)

From: Kathy Strawn [mailto:mstrawn@olemiss.edu]
Sent: Monday, May 10, 2010 9:06 AM
To: Pawar, Rahul
Subject: RE: Happy mothers day

You are so sweet! I hope (b) (6) had a nice one! My (b) (6) from (b) (6) came Saturday afternoon and we had a wonderful, fun afternoon of shopping on the Square. It was commencement week-end and there was a baseball game and the Square was FULL of people. Then of course, we went to brunch on Sunday and everywhere was packed. But it was wonderful to get to hang out with her. Lori was traveling in Washington (the state) and I missed getting to be with her.

Thanks for the sweet email and for staying in touch. Give (b) (6) and little (b) (6) my love.

Kathy Strawn
Senior Administrative Secretary
National Center for Natural Products Research

P.O. Box 1848
University, MS 38677
Phone (662) 915-1090
Fax (662) 915-7989
Email mstrawn@olemiss.edu

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Monday, May 10, 2010 7:58 AM
To: mstrawn@olemiss.edu
Subject: Happy mothers day

Hi Kathy,
Wish you a very happy Mothers Day.
Rahul, (b) (6)

From: [Pawar, Rahul](#)
To: ["mstrawn@olemiss.edu"](mailto:mstrawn@olemiss.edu)
Subject: RE: Happy mothers day
Date: Tuesday, May 11, 2010 11:19:42 AM

Thank you Dear,
Nothing to worry. He called me today in the morning. Fedex will be good
Thank you and have a good day
Rahul

From: Kathy Strawn [mailto:mstrawn@olemiss.edu]
Sent: Tuesday, May 11, 2010 10:50 AM
To: Pawar, Rahul
Subject: RE: Happy mothers day

I was worried I had dropped the ball myself on your recommendation letters, but Dr. Khan had not forwarded the drafts to me. So here they are. I will send the originals by mail, or do I need to Fed Ex them to you.

Kathy Strawn
Senior Administrative Secretary
National Center for Natural Products Research
P.O. Box 1848
University, MS 38677
Phone (662) 915-1090
Fax (662) 915-7989
Email mstrawn@olemiss.edu

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Tuesday, May 11, 2010 8:22 AM
To: mstrawn@olemiss.edu
Subject: RE: Happy mothers day

Good morning Kathy,
Do you know, if Dr. Khan prepared and mailed my recommendation letter?
Please let me know
Thanks
Rahul

From: Kathy Strawn [mailto:mstrawn@olemiss.edu]
Sent: Monday, May 10, 2010 9:15 AM
To: Pawar, Rahul
Subject: RE: Happy mothers day

That's great. Tell (b) (6) hello for me.

Kathy Strawn
Senior Administrative Secretary
National Center for Natural Products Research
P.O. Box 1848
University, MS 38677
Phone (662) 915-1090
Fax (662) 915-7989

Email mstrawn@olemiss.edu

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Monday, May 10, 2010 8:10 AM
To: mstrawn@olemiss.edu
Subject: RE: Happy mothers day

I am Happy you had a great day.

(b) (6) had a good one too, with lots of gifts and a card from (b) (6).

From: Kathy Strawn [mailto:mstrawn@olemiss.edu]
Sent: Monday, May 10, 2010 9:06 AM
To: Pawar, Rahul
Subject: RE: Happy mothers day

You are so sweet! I hope (b) (6) had a nice one! My (b) (6) from (b) (6) came Saturday afternoon and we had a wonderful, fun afternoon of shopping on the Square. It was commencement week-end and there was a baseball game and the Square was FULL of people. Then of course, we went to brunch on Sunday and everywhere was packed. But it was wonderful to get to hang out with her. (b) (6) was traveling in Washington (the state) and I missed getting to be with her.

Thanks for the sweet email and for staying in touch. Give (b) (6) and little (b) (6) my love.

Kathy Strawn
Senior Administrative Secretary
National Center for Natural Products Research
P.O. Box 1848
University, MS 38677
Phone (662) 915-1090
Fax (662) 915-7989
Email mstrawn@olemiss.edu

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Monday, May 10, 2010 7:58 AM
To: mstrawn@olemiss.edu
Subject: Happy mothers day

Hi Kathy,
Wish you a very happy Mothers Day.
Rahul, (b) (6)

From: [Pawar, Rahul](#)
To: ["BHARATHI AVULA"](#)
Subject: RE: Happy New Year
Date: Friday, January 18, 2013 10:08:36 AM
Attachments: [s18.pdf](#)

Hi Bharathi, Happy new year to you too,
How are you all doing?
Where are you? I called you two times last week,
Rahul

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Friday, January 18, 2013 10:01 AM
To: Pawar, Rahul
Subject: Happy New Year

Happy new year to you all...

I hope everything is going well from your side...

Best wishes
Bharathi

NOTE: Mail me the following paper:

[Quantitative determination of alkaloids from roots of Hydrastis canadensis L. and dietary supplements using ultra-performance liquid chromatography with UV detection:](#)
Avula, Bharathi; Wang, Yan-Hong; Khan, Ikhlas A. Journal of AOAC International (2012), 95(5), 1398-1405

From: [Pawar, Rahul](#)
To: ["bavula@olemiss.edu"](mailto:bavula@olemiss.edu)
Subject: RE: Happy New Year
Date: Friday, January 7, 2011 12:21:00 PM

Happy new year!
We are back yesterday. The trip was grea (b) (6) ad a great time in India.
How was your holidays?
Talk to you soon
Rahul

From: bavula@olemiss.edu [mailto:bavula@olemiss.edu]
Sent: Friday, January 07, 2011 11:15 AM
To: Pawar, Rahul
Subject: Happy New Year

Are you back from India???

I hope you all had a good time....

Happy New Year
Bharathi

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: RE: Happy New Year
Date: Friday, January 18, 2013 10:09:36 AM

Thank you...

I am sorry I missed your call...

Best wishes

Bharathi

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Friday, January 18, 2013 9:09 AM
To: BHARATHI AVULA
Subject: RE: Happy New Year

Hi Bharathi, Happy new year to you too,
How are you all doing?
Where are you? I called you two times last week,
Rahul

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Friday, January 18, 2013 10:01 AM
To: Pawar, Rahul
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Happy new year to you all...

I hope everything is going well from your side...

Best wishes

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Avula, Bharathi; Wang, Yan-Hong; Khan, Ikhlas A. Journal of AOAC International (2012), 95(5), 1398-1405

From: [Pawar, Rahul](#)
To: [Ikhlas Khan](#)
Subject: RE: Hello
Date: Thursday, August 13, 2015 3:55:00 PM

Ok, I will ask for the list to be prepared.

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Thursday, August 13, 2015 3:54 PM
To: Pawar, Rahul
Cc: BHARATHI AVULA
Subject: Re: Hello

Rahul
Just send the list of the products you have

Sent from my iPhone

On Aug 13, 2015, at 3:08 PM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov> wrote:

Thank you Bharathi, will check how many products we have for these.

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Thursday, August 13, 2015 3:06 PM
To: Pawar, Rahul
Cc: Ikhlas Khan
Subject: FW: Hello

we have methods for following:

Momordica charantia (karela)
Cinnamon Spp
Morus rubra

thanks
bharathi

From: Ikhlas Khan
Sent: Thursday, August 13, 2015 1:44 PM
To: BHARATHI AVULA <bavula@olemiss.edu>
Subject: Fwd: Hello

Fyi

Sent from my iPhone

Begin forwarded message:

From: "Pawar, Rahul" <Rahul.Pawar@fda.hhs.gov>
Date: August 13, 2015 at 2:25:14 PM EDT
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Hello

Dr. Khan,
Nice meeting you here.
Can you please tell me which methods you already have developed for the antidiabetic ingredients. See ingredient list below
Thanks
Rahul

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Wednesday, March 04, 2015 4:18 PM
To: Pawar, Rahul
Cc: BHARATHI AVULA
Subject: Re: Hello

Good, we do have some methods ready and some we will develop.
ik

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Wed, 4 Mar 2015 20:43:45 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Hello

We have a list of about 50-60 products (we have not purchased them yet). Most products are combination of the ingredients in the list.

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Wednesday, March 04, 2015 3:28 PM
To: Pawar, Rahul
Cc: BHARATHI AVULA
Subject: Re: Hello

How many total products and how many in each category

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Wed, 4 Mar 2015 18:23:30 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Hello

Hello Dr. Khan,
Here is the list of botanicals we intend to target for selection of antidiabetic/sugar balance DS products.

Mormordica charantia (karela)
[Gymnema Sylvestre](#). (Gurmar)

Trigonella foenum (Fenugreek Seed)
Cinnamon Sp.
Occimum spp. (Holy Basil Leaf)
Irvingia gabonensis (African Mango)
Eugenia jambolina (Jamun/Jambul)
Lagestroemia speciosa (Banaba leaf)
White Mulberry leaf (Morus)

Let me know your opinion and any possible additions/deletions to this. You have methods for how many of these ingredients?

Best wishes
Rahul

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Thursday, February 12, 2015 11:51 AM
To: Pawar, Rahul
Subject: Re: Hello

Sorry, I am in Jamnagar for couple of days before go to Kolkata. I will be happy to discuss and happy to hear that he agrees. On teleconference sounded like he does not want to mix two things together.
Anyway we will talk some more.
IK

From: <Pawar>, Rahul Pawar <Rahul.Pawar@fda.hhs.gov>
Date: Wednesday, February 11, 2015 at 3:24 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Hello

Hello Dr. Khan.
I tried to call you couple of times and then came to know from Bharathi that you are travelling to India. Congratulations for the award!
I spoke with my manager and there is positive response for starting an antidiabetic botanical supplement project with NCNPR. Hope to talk with you more when you are back.
Best regards
Rahul

Rahul Pawar Ph.D.
Office of Regulatory Science
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-3077

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Cc: [BHARATHI AVULA](#)
Subject: Re: Hello
Date: Wednesday, March 4, 2015 4:17:52 PM

Good, we do have some methods ready and some we will develop.
ik

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Wed, 4 Mar 2015 20:43:45 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Hello

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White Mulberry leaf (Morus)

Let me know you opinion and any possible additions/deletions to this. You have methods for how many of these ingredients?

Best wishes

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Sent: Thursday, February 12, 2015 11:51 AM
To: Pawar, Rahul
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IK

From: <Pawar>, Rahul Pawar <Rahul.Pawar@fda.hhs.gov>
Date: Wednesday, February 11, 2015 at 3:24 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Hello

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Best regards

Rahul

Rahul Pawar Ph.D.
Office of Regulatory Science
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-3077

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE: Hello
Date: Thursday, February 12, 2015 12:01:00 PM

Good to hear from you. Yes, we will discuss after you are back.
I thought you are attending Kejriwal's inaugurations!!
Safe travel
Rahul

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Thursday, February 12, 2015 11:51 AM
To: Pawar, Rahul
Subject: Re: Hello

Sorry, I am in Jamnagar for couple of day before go to kolkata. I will be happy to discuss and happy to hear that he agrees. On teleconference sounded like he does not want to mix two things together.
Anyway we will talk some more.
IK

From: <Pawar>, Rahul Pawar <Rahul.Pawar@fda.hhs.gov>
Date: Wednesday, February 11, 2015 at 3:24 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Hello

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5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-3077

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: RE: Hello
Date: Friday, February 7, 2014 12:32:22 AM

mom is (b) (6) .

thank you for the wishes

bharathi

From: Pawar, Rahul [Rahul.Pawar@fda.hhs.gov]
Sent: Thursday, February 06, 2014 8:05 AM
To: BHARATHI AVULA
Subject: RE: Hello

Hi Bharathi,

That is what I thought. It is good to know aunty is getting (b) (6) . Sure there will be discomfort but she will be better in few days. I am also glad you are there with them.

Convey our regards and love to both uncle and aunty as ask her to take it easy.

Rahul

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Wednesday, February 05, 2014 10:44 PM
To: Pawar, Rahul
Subject: RE: Hello

I am still in India and will be back on 20th of this month.

Mom had (b) (6) taking care of her.

She is doing okay but not good

bharathi

From: Pawar, Rahul [Rahul.Pawar@fda.hhs.gov]
Sent: Tuesday, February 04, 2014 9:46 AM
To: BHARATHI AVULA
Subject: Hello

Hi Bharathi,

Hope you are back, How was your vacations?

Will talk to you soon.

Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740

Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE: Hello
Date: Thursday, September 19, 2013 9:01:00 AM

Ok, thanks

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Thursday, September 19, 2013 9:00 AM
To: Pawar, Rahul
Subject: Re: Hello

Ok, I will get in touch afternoon since its not clear what we will do tonight but if I have time I will come by, don't worry about dinner.
IK

From: Rahul Pawar <Rahul.Pawar@fda.hhs.gov>
Date: Thu, 19 Sep 2013 12:58:04 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Hello

Sorry sir, I am with (b) (6) !

Why don't you come over for dinner, I will pick you up, it is 10 min drive.

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Thursday, September 19, 2013 8:53 AM
To: Pawar, Rahul
Subject: Re: Hello

You can join us for lunch if you can.
Ik

From: Rahul Pawar <Rahul.Pawar@fda.hhs.gov>
Date: Thu, 19 Sep 2013 12:39:21 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Hello

Great, No in in fact closer. Let me know if you have time. I am working from home today.
Are you also attending the next weeks' USP botanical adulteration workshop? I am.

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Thursday, September 19, 2013 8:36 AM
To: Pawar, Rahul
Subject: Re: Hello

I am in baltimore marriot for usp conference. Good to hear that it worked out for Li, thanks
I assume baltimore is far from your place

Sent from my iPhone

On Sep 19, 2013, at 8:30 AM, "Pawar, Rahul" <Rahul.Pawar@fda.hhs.gov> wrote:

Hello Sir,

I heard you are in DC.

Just want to update that Jing li will be joining us in January 14.

Thanks

Rahul

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE: Hello
Date: Wednesday, August 21, 2013 11:44:29 PM

Sir, Two of your posters are on 28 morning. I could not locate the third one, what the name of the first author?

Mr. Sandu is currently in Washington DC. If you are interested in meeting him I can give his phone number.

Please try to spare some time to visit us, Hema's mummy make great mutton Korma!

Let me know if you need anything during your visit.

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Wednesday, August 21, 2013 3:55 PM
To: Pawar, Rahul
Subject: Re: Hello

Yes we are attending it. Arriving on Sunday

Sent from my iPhone

On Aug 21, 2013, at 2:16 PM, "Pawar, Rahul" <Rahul.Pawar@fda.hhs.gov> wrote:

Hello Dr. Khan,
I just called you a minute ago to check if you are attending the FDA conference next week.
Hope you all are doing well. Say our hi to [REDACTED]
Talk to you soon. (b) (6)
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: ["mstrawn@olemiss.edu"](mailto:mstrawn@olemiss.edu)
Subject: RE: Hi
Date: Wednesday, April 28, 2010 4:18:08 PM

Dear Kathy,
You really make us feel bad about leaving oxford. Thank you for all the love.
I am happy you are retiring, I am sure you will have good time. We wish you all the best and good health.
(b) (6) likes the monkey you gave her and she always remember you.
My cell is (b) (6) and email is (b) (6)
I am planning to change my residence and will update it later.
Stay in touch
Best regards
Rahul

From: Kathy Strawn [mailto:mstrawn@olemiss.edu]
Sent: Wednesday, April 28, 2010 9:31 AM
To: Pawar, Rahul
Subject: RE: Hi

Hello! It was wonderful seeing you all. (b) (6) is just so adorable and you all make a beautiful family. I still am smiling over getting to visit with you.

My official retirement date is May 31. I gave my letter to Dr. Khan last Friday. I may take a few days off here and there. It seems strange to think I really will be retiring. It will be hard to finish out the time knowing I'm leaving. But the time will pass quickly.

My home address is: (b) (6) I use my cell for my home phone and it i (b) (6) lease keep in touch.

I need to update Dr. Khan's CV with some publications and meetings, but here it is.

Tell (b) (6) hello for me and kiss (b) (6) and remind her I like her!

Kathy Strawn
Senior Administrative Secretary
National Center for Natural Products Research
P.O. Box 1848
University, MS 38677
Phone (662) 915-1090
Fax (662) 915-7989
Email mstrawn@olemiss.edu

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Wednesday, April 28, 2010 8:12 AM
To: mstrawn@olemiss.edu
Subject: Hi

Dear Kathy,
I hope you are doing well.

It was wonderful to see you at oxford, I am so glad we all could make it to oxford.

By the way when are you retiring?

Can you please do me a favor, I want a recent CV of Dr Khan for drafting a recommendation letter.

Please send one when you have time.

Sorry, I will send the pictures to you soon.

Also please send me your personal e-mail/phone number

Best Regards

Rahul

Rahul Pawar Ph.D.

Division of Bioanalytical Chemistry

CFSAN-FDA

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 301-436-1795

Fax: 301-436-2622

From: bavula@olemiss.edu
To: [Pawar, Rahul](#)
Subject: Re: Hoodia-ABC herbclip
Date: Wednesday, May 19, 2010 5:48:34 PM

Thank you Rahul

Bharathi

On Wednesday 05/19/2010 at 12:39 pm, "Pawar, Rahul" wrote:

<<Hoodia-ABC Herbclip.pdf>>

FYI

From: [Pawar, Rahul](#)
To: ["bavula@olemiss.edu"](mailto:bavula@olemiss.edu)
Subject: RE: Hoodigogenin A Paper
Date: Friday, January 15, 2010 9:53:55 AM

Thanks, I saw it earlier
Good day

From: bavula@olemiss.edu [mailto:bavula@olemiss.edu]
Sent: Friday, January 15, 2010 8:55 AM
To: yatin shukla; Pawar, Rahul
Subject: Hoodigogenin A Paper

Please find enclosed the paper published in Planta Medica.

Thank you
Bharathi

From: [Pawar, Rahul](#)
To: ["BHARATHI AVULA"](#)
Subject: RE: House
Date: Tuesday, February 21, 2012 9:38:32 AM

Congratulations!!!! Send some pictures if you have. Will call you today. Let us know if we can be of any help

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Monday, February 20, 2012 8:29 AM
To: Pawar, Rahul
Subject: House

Dear Rahul and Hema,

My (b) (6) almost finalized...By this weekend I will confirm please.....

Thank you for all your support

Bharathi

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Re: <http://cen.acs.org/articles/92/i39/Tramadols-Newfound-Natural-Product-Status.html>
Date: Thursday, October 2, 2014 3:57:28 PM

Thanks, at least it will stop new products coming to market.
IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Thu, 2 Oct 2014 19:45:26 +0000
To: Ikhlas Khan <ikh@olemiss.edu>
Subject: <http://cen.acs.org/articles/92/i39/Tramadols-Newfound-Natural-Product-Status.html>

Interesting story, hope you know by now.

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
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5100 Paint Branch Parkway
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Tel: 240-402-1795
Fax: 301-436-2622

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Re: http://www.bizjournals.com/prnewswire/press_releases/2015/04/29/CL93423
Date: Thursday, April 30, 2015 11:18:52 AM

Interesting
ik

From: <Pawar>, Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Thursday, April 30, 2015 at 9:35 AM
To: Ikhlas Khan <ikh@olemiss.edu>
Subject: http://www.bizjournals.com/prnewswire/press_releases/2015/04/29/CL93423

Rahul Pawar, Ph.D.
Office of Regulatory Science
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5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-3077

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE: ICSB coverage from Nutraingredients-usa.com
Date: Saturday, April 16, 2016 12:22:00 AM

Yes, I read it at the Memphis airport. Mark also showed me his comments couple of days back.
Thank you for the opportunity to present our work.
As always great to meet you all and spend some time.

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Friday, April 15, 2016 11:55 AM
To: Pawar, Rahul
Subject: FW: ICSB coverage from Nutraingredients-usa.com

Told you so
ik

From: "frank@unpa.com" <frank@unpa.com>
Date: Friday, April 15, 2016 at 10:35 AM
To: Loren Israelsen <loren@unpa.com>
Cc: Ikhlas Khan <ikhlan@olemiss.edu>, Peter Reinecke <peter@rssinc.us>, Patricia Knight <pknight@knight-cap.us>
Subject: ICSB coverage from Nutraingredients-usa.com

Data confirms that DNA barcoding alone not suitable for finished dietary supplement products: http://www.nutraingredients-usa.com/Research/Data-confirms-that-DNA-barcoding-alone-not-suitable-for-finished-dietary-supplement-products/?utm_source=newsletter_daily&utm_medium=email&utm_campaign=15-Apr-2016&c=6htVoOGdDM2Ft9cj6P41qV9epLetwIXu&p2=

FDA 'appreciates' CRN's product registry efforts/Sharfstein presentation:
http://www.nutraingredients-usa.com/Regulation/FDA-appreciates-CRN-s-product-registry-efforts/?utm_source=newsletter_daily&utm_medium=email&utm_campaign=15-Apr-2016&c=6htVoOGdDM0ER4G00d4eyX%2Fy72wRbm9o&p2=

From: [Pawar, Rahul](#)
To: [Jennifer S. Taylor](#)
Subject: RE: ICSB speaker request
Date: Tuesday, March 15, 2016 8:30:00 AM

Sure, will send it soon...

From: Jennifer S. Taylor [mailto:jnnfrtyl@olemiss.edu]
Sent: Monday, March 14, 2016 5:36 PM
To: Pawar, Rahul
Subject: ICSB speaker request

Dr. Pawar,

Can you please send me a one page biosketch for the booklet. I would like to finish the booklet this week.

Thanks,

Jennifer Taylor

Jennifer Taylor
Program Coordinator
University of Mississippi
National Center for Natural Products Research
3012 Thad Cochran
P.O. Box 1848
University, MS 38677
 jnnfrtyl@olemiss.edu
 662-915-1090
 662-915-7989

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From: [Pawar, Rahul](#)
To: [Amar Chittiboyina](#)
Subject: RE: ICSB
Date: Tuesday, December 6, 2016 10:49:00 AM

Np..

From: Amar Chittiboyina [mailto:amar@olemiss.edu]
Sent: Tuesday, December 06, 2016 10:30 AM
To: Pawar, Rahul
Subject: RE: ICSB

Sorry Rahul for not able to get back to you earlier. Thanks, Amar

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Tuesday, December 06, 2016 8:59 AM
To: AMAR GOPAL CHITTIBOYINA
Subject: RE: ICSB

3rd Jan, thanks!

From: AMAR GOPAL CHITTIBOYINA [mailto:amar@olemiss.edu]
Sent: Friday, December 02, 2016 3:02 PM
To: Pawar, Rahul
Subject: Re: ICSB

Probably. But we haven't decided anything at this point. Shall keep you posted.
Amar

Sent from Amar's iPhone

On Dec 2, 2016, at 1:49 PM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov> wrote:

Hi Amar,
Hope all is well. Are there any plans for extending the deadline for abstract submission?
Thanks,
Rahul

Rahul Pawar, Ph.D.
Research Chemist
Office of Regulatory Science/DBC/BMB
FDA/Center for Food Safety and Applied Nutrition
5001 Campus Drive
College Park, MD 20740
Tel: 240-402-1795

From: Jennifer S. Taylor
To: [Pawar, Rahul](#)
Subject: RE: information request
Date: Thursday, September 6, 2012 3:38:38 PM

The list of names that email was sent to. If you do reply all you will see them.

Jennifer Taylor

Jennifer Taylor
Senior Secretary
University of Mississippi
National Center for Natural Products Research
301Q Thad Cochran
P.O. Box 1848
University, MS 38677

✉ jnnfrtyl@olemiss.edu
☎ 662-915-1090
📠 662-915-7989

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Thursday, September 06, 2012 12:06 PM
To: Jennifer S. Taylor
Subject: RE: information request

I don't see any list attached

From: Jennifer S. Taylor [<mailto:jnnfrtyl@olemiss.edu>]
Sent: Thursday, September 06, 2012 12:01 PM
To: Atish Paul; Atul Jadhav; chidananda swamy Rumalla; Dr. Vijai K Agnihotri; earla ravinder; Ehab; Erdal Bedir; Feng Wei; Hyung-in Moon (himun68@dau.ac.kr); Jamal Mustafa; jamal mustafa mustafa; Julius Ngunde Ngwendson; li jing; MATSUZAKI Keiichi (matsuzaki.keiichi@nihon-u.ac.jp); Nurdan S Duzgoren-Aydin (naydin@njcu.edu); Pawar, Rahul; Sara Crockett; Sridhar Rao Ayinampudi; Toshiaki Makino (makino@phar.nagoya-cu.ac.jp); Vaishali Joshi; vamsi.m; wangwei; yatin shukla; Young-Whan Choi (ywchoi@pusan.ac.kr)
Cc: Ikhlas Khan
Subject: information request

Dr. Khan would like to have your current contact information along with your current employment information. He is working on a project and needs to have this info. Also, if you happen to know any contact info for other that do not appear on my list, please provide it for me so that I can have a complete list of past group members. Thank you for your time and cooperation.

Jennifer Taylor

Jennifer Taylor
Senior Secretary
University of Mississippi
National Center for Natural Products Research
301Q Thad Cochran
P.O. Box 1848
University, MS 38677

✉ jnnfrtyl@olemiss.edu

☎ 662-915-1090

📠 662-915-7989

From: Betsy_Baer@waters.com
To: [Ikhlas Khan](#)
Cc: [Kate_Yu@waters.com](#); [Pawar, Rahul](#)
Subject: Re: Invitation: TC with Ikhlas Khan (Dial (b) (6) , access code (b) (6)) (Mar 3 04:00 PM EST)
Date: Tuesday, March 3, 2015 6:04:50 PM

Thank you so much Ikhlas for the information and for introducing me to Rahul. It was a pleasure speaking with you and I look forward to seeing you in April. Have a good night.

All the best,
Betsy

Sent from my iPhone

On Mar 3, 2015, at 4:37 PM, "Ikhlas Khan" <ikhlan@olemiss.edu> wrote:

RAHUL PAWAR
Rahul Pawar <Rahul.Pawar@fda.hhs.gov>

CFSAN PERSON
IK

From: "Kate_Yu@waters.com" <Kate_Yu@waters.com>
Date: Saturday, February 28, 2015 at 8:37 PM
To: <Betsy_Baer@waters.com>, Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Invitation: TC with Ikhlas Khan (Dial in (b) (6) , access code (b) (6)) (Mar 3 04:00 PM EST)

Description

Dial in number

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Dial in number

===== The information in this email is confidential, and is intended solely for the addressee(s). Access to this email by anyone else is unauthorized and therefore prohibited. If you are not the intended recipient you are notified that disclosing, copying, distributing or taking any action in reliance on the contents of this information is strictly prohibited and may be unlawful.

=====

From: [Pawar, Rahul](#)
To: ["BHARATHI AVULA"](#)
Subject: RE: JMS
Date: Friday, April 5, 2013 8:10:12 AM

Hello Mam,
Send me the reference I will try.
I am not coming for the meeting and also to ASP this year. Budget is tight for travel. I have a talk at ACS meeting at new Orleans this Monday

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Friday, April 05, 2013 8:07 AM
To: Pawar, Rahul
Subject: JMS

Do you by any chance have access to Journal of Mass Spectrometry????

Are you coming for our upcoming conference??

bharathi

From: [Pawar, Rahul](#)
To: ["bavula@olemiss.edu"](mailto:bavula@olemiss.edu)
Subject: RE: Journal Paper
Date: Monday, June 28, 2010 3:52:20 PM

yes, looks like that is a typo. Good eyes!

From: bavula@olemiss.edu [mailto:bavula@olemiss.edu]
Sent: Monday, June 28, 2010 3:22 PM
To: Pawar, Rahul
Subject: RE: Journal Paper

Sorry for bothering...

On page # 381, it says "named as bacopasaponin G" (is it bacopasaponin H-page #382)

Thank you
Bharathi

On Monday 06/28/2010 at 1:51 pm, "Pawar, Rahul" wrote:

-----Original Message-----

From: bavula@olemiss.edu [mailto:bavula@olemiss.edu]
Sent: Monday, June 28, 2010 2:50 PM
To: Pawar, Rahul
Subject: Journal Paper

Could you please email me your journal paper:

Pawar, Rahul S., Khan, Shabana I., Khan, Ikhlas A.:
Glycosides of 20-Deoxy Derivatives of Jujubogenin and
Pseudojujubogenin from Bacopa monniera

Thank you
Bharathi

From: [Pawar, Rahul](#)
To: ["BHARATHI AVULA"](#)
Subject: RE: Kratom
Date: Thursday, October 16, 2014 9:39:00 AM

Great thanks!

-----Original Message-----

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Thursday, October 16, 2014 9:33 AM
To: Pawar, Rahul
Subject: RE: Kratom

one day depending on instrument behavior

bharathi

From: Pawar, Rahul [Rahul.Pawar@fda.hhs.gov]
Sent: Thursday, October 16, 2014 8:26 AM
To: BHARATHI AVULA
Subject: RE: Kratom

If you have the sample how long will it take for you to have the answer? Thanks Best Rahul

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Thursday, October 16, 2014 9:19 AM
To: Pawar, Rahul
Cc: ZULFIQAR ALI; Ikhlas Khan
Subject: RE: Kratom

OK....Rahul..

It is in the pipeline but did not start yet...

We will let you know after analysis is done

Have a good day
bharathi

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Thursday, October 16, 2014 8:14 AM
To: BHARATHI AVULA
Cc: ZULFIQAR ALI; Ikhlas Khan
Subject: RE: Kratom

Thanks you Bharathi,

I thought you already have worked on this. Yes, got this 1967 paper on M Javanica not sure if anything was done recently. Let me know if you come across something.

Best

Rahul

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Thursday, October 16, 2014 8:56 AM
To: Ikhlas Khan

Cc: ZULFIQAR ALI; Pawar, Rahul
Subject: RE: Kratom

Yes Sir....I will check with Vijay

bharathi

From: Ikhlas Khan
Sent: Thursday, October 16, 2014 7:54 AM
To: BHARATHI AVULA; Pawar, Rahul (Rahul.Pawar@fda.hhs.gov<<mailto:Rahul.Pawar@fda.hhs.gov>>)
Cc: ZULFIQAR ALI
Subject: Re: Kratom

We need to get the sample to confirm it.
ik

From: Bharathi Avula <bavula@olemiss.edu<<mailto:bavula@olemiss.edu>>>
Date: Thursday, October 16, 2014 at 7:01 AM
To: Rahul Pawar <Rahul.Pawar@fda.hhs.gov<<mailto:Rahul.Pawar@fda.hhs.gov>>>
Cc: Ikhlas Khan <ikhan@olemiss.edu<<mailto:ikhan@olemiss.edu>>>, ZULFIQAR ALI <zulfiqar@olemiss.edu<<mailto:zulfiqar@olemiss.edu>>>
Subject: FW: Kratom

Dear Rahul,

From literature search, the M. javanica did not contain mitragynine (C₂₃H₃₀N₂O₄; 398.2205) but contains javaphylline (C₂₂H₂₆N₂O₅; 398.1841), mitrajavine...

Thank you
bharathi

From: Ikhlas Khan
Sent: Thursday, October 16, 2014 3:55 AM
To: BHARATHI AVULA; ZULFIQAR ALI
Subject: Fwd: Kratom

Can you respond to rahul

Sent from my iPhone

Begin forwarded message:
From: "Pawar, Rahul" <Rahul.Pawar@fda.hhs.gov<<mailto:Rahul.Pawar@fda.hhs.gov>>>
Date: October 16, 2014 at 4:30:52 AM GMT+8
To: "ikhan@olemiss.edu<<mailto:ikhan@olemiss.edu>>" <ikhan@olemiss.edu<<mailto:ikhan@olemiss.edu>>>
Subject: Kratom
Hello Sir,
One quick question. Does Mitragyna javanica contain Mitragynine?
Thanks
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795

From: [Pawar, Rahul](#)
To: [Ikhlas Khan](#)
Cc: [JULIE R MIKELL](#); [Jennifer S. Taylor](#)
Subject: RE: Lab supplies
Date: Thursday, October 2, 2014 3:43:00 PM

Good, One thing I forgot to mention that the person doing the paper work and the recipient on the shipping label must be same. I will send the forms to Julie and Jennifer.

Rahul

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Thursday, October 02, 2014 3:39 PM
To: Pawar, Rahul
Cc: JULIE R MIKELL; Jennifer S. Taylor
Subject: Re: Lab supplies

Hi Rahul

Thanks for your offer. Jennifer will send you shipping# and information and Julie can take care of material and whatever needs to be done since she has been Biotage person.

Thanks

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Thu, 2 Oct 2014 19:32:54 +0000
To: Ikhlas Khan <ikhan@olemiss.edu>
Subject: Lab supplies

Hello Dr. Khan,

Forgot to talk with you about shipping out the Biotage columns. Please ask Amar or somebody with whom I can communicate to do the paper work.

As I said, you will have to pay for the shipment. I will put them in one or two boxes and put **your fedex/UPS label** for shipment. There are few forms that the receiving person needs to fill but it is fairly simple.

I can ship

1. Biotage SNAP Silica 50 g- 30 columns
2. Biotage SNAP Silica 100 g- 30 columns
3. Biotage SNAP Silica 340 g- 4 columns
4. Biotage SNAP Silica 750 g- 4 columns
5. Biotage SNAP Silica 1500g- 2 columns
6. Biotage SNAP C18 120g- 2 columns
7. Biotage Dry load vessels-4
8. I might also have some smaller C18 columns

I also have Chem Elut 300 ml cartridges (Agilent) very good for alkaloid enrichment or extract clean-up- 60 columns

Let me know if this works. Thanks

Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: ["JULIE R MIKELL"](#)
Subject: RE: Lab supplies
Date: Monday, October 6, 2014 10:26:00 AM
Attachments: [DonationPackageCPK2091914.pdf](#)

Hi Julie,

Can you please send me the signed letter of request (on the NCNPR or UM letterhead) and the completed EO12999. Page 5 and 7 on the pdf file only) Thanks

Rahul

240-402-1795

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Thursday, October 02, 2014 3:39 PM
To: Pawar, Rahul
Cc: JULIE R MIKELL; Jennifer S. Taylor
Subject: Re: Lab supplies

Hi Rahul

Thanks for your offer. Jennifer will send you shipping# and information and Julie can take care of material and whatever needs to be done since she has been Biotage person.

Thanks

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Thu, 2 Oct 2014 19:32:54 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Lab supplies

Hello Dr. Khan,

Forgot to talk with you about shipping out the Biotage columns. Please ask Amar or somebody with whom I can communicate to do the paper work.

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7. Biotage Dry load vessels-4
8. I might also have some smaller C18 columns

I also have Chem Elut 300 ml cartridges (Agilent) very good for alkaloid enrichment or extract clean-up- 60 columns

Let me know if this works. Thanks
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: Jennifer Michael
To: [Pawar, Rahul](#)
Subject: RE: Letter for Rahul Pawar
Date: Tuesday, May 4, 2010 12:46:57 PM

Okay good. That should give me time to update it. He will be leaving on vacation tomorrow, so I will get to it this week. Have a good rest of the week.

Jennifer

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Tuesday, May 04, 2010 11:33 AM
To: Jennifer Michael
Subject: RE: Letter for Rahul Pawar

[anytime early next week is ok](#)

From: Jennifer Michael [mailto:jenmike@olemiss.edu]
Sent: Tuesday, May 04, 2010 12:31 PM
To: Pawar, Rahul
Subject: RE: Letter for Rahul Pawar

I am supposed to be updating Dr. Walker's CV and have not yet completed it. How soon do you need it? Sorry, I had already closed up the envelope.

Jennifer

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Tuesday, May 04, 2010 11:27 AM
To: Jennifer Michael
Subject: RE: Letter for Rahul Pawar

Hi Jennifer,

I came to know about your daughters remarkable achievements from (b) (6)

Please send me a copy of Dr Walker's CV, if you have already closed the envelope than electronic copy is fine

Thank you

Rahul

From: Jennifer Michael [mailto:jenmike@olemiss.edu]
Sent: Tuesday, May 04, 2010 11:44 AM
To: Pawar, Rahul
Subject: RE: Letter for Rahul Pawar

I am doing very well....despite the fact my only child/daughter (b) (6) this month!!! Yikes! I will get the letter in the mail to you today. I enjoyed visiting with (b) (6) and your precious daughter!

Take care.

Jennifer Michael

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Tuesday, May 04, 2010 10:37 AM
To: Jennifer Michael
Subject: RE: Letter for Rahul Pawar

Hi Jennifer,
I hope you are doing well
Thank you for the timely help.
Yes, please send the letters by mail to the following address.
Please convey my thank you and regards to Dr. Walker.
Best regards
Rahul

*Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway (HFS-717)
College Park, MD 20740*

*Tel: 301-436-1795
Fax: 301-436-2622*

From: Jennifer Michael [mailto:jenmike@olemiss.edu]
Sent: Tuesday, May 04, 2010 11:32 AM
To: Pawar, Rahul
Cc: lwalker@olemiss.edu
Subject: FW: Letter for Rahul Pawar

Rahul:

Here is the letter Dr. Walker prepared for you. Please let me know if you need me to mail the original and what address it needs to be sent to.

Thank you.

**Jennifer W. Michael, Staff Assistant
National Center for Natural Products Research
University of Mississippi
P. O. Box 1848
University, MS 38677
Phone: 662-915-1005
Fax: 662-915-1006
Email: jenmike@olemiss.edu**

From: [Pawar, Rahul](#)
To: ["bavula@olemiss.edu"](mailto:bavula@olemiss.edu)
Subject: RE: letter format for reviewing the papers
Date: Monday, January 31, 2011 10:41:00 AM

Sorry, I was out of office for a week with no access to my office mails, just got back.

Each evidence will have a number (Exhibit no). You should give each reco letter a number, when you discuss about it in your petition letter use that number (kind of reference number you of in papers)

Foe editors letter---Some thing like this will be good, Get from all the editors you work for, more the better....

-

To whom so ever it may concern,

This letter certifies that Dr. Rahul Pawar have been an active reviewer for our journal, Planta Medica, since April 2005. Planta Medica is one of the leading international journals in the field of medicinal plants and natural products and publishes original research findings from researchers worldwide. Planta Medica publishes 15 issues per year and covers the areas of pharmacology and clinical studies, natural product chemistry, analytical studies, and biological screening of medicinal plants and natural products.

Dr. Pawar is an expert in natural product chemistry and has reviewed 11 scientific papers for our journal of which 3 of them in the past 12 months.

We appreciate his contribution to our journal very much!

Editor

Hope this helps
Rahul

From: bavula@olemiss.edu [<mailto:bavula@olemiss.edu>]
Sent: Friday, January 28, 2011 8:46 AM
To: Pawar, Rahul
Subject: letter format for reviewing the papers

Could you please email me the matter should be presented in the letter for reviewing the papers...

I can ask the JAOAC editor to give me one please

Do we need from other journals also, or one is enough...

Thank you
Bharathi

From: [Pawar, Rahul](#)
To: [NCNPR](#)
Subject: RE: Link to Account Approved
Date: Tuesday, November 4, 2014 12:32:00 PM

240-402-1795

Forgot a big box, there are 8 in total

Approx. weights are

Box 1- 9 lb

Box 2- 15 lb

Box 3- 9 lb

Box 4- 7 lb

Box 5- 10 lb

Box 6- 9 lb

Box 7-40 lb

Box 8- 10 lb

Thanks

Rahul

From: NCNPR [mailto:ncnpr@olemiss.edu]
Sent: Tuesday, November 04, 2014 11:46 AM
To: Pawar, Rahul
Cc: Jennifer S. Taylor
Subject: RE: Link to Account Approved

I will need a telephone number in order to process the labels.

So you are needing 7 separate shipping labels? I will need to know how much each of the seven boxes weigh; cannot use the total of 100 on one box.
Jennifer Michael

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Tuesday, November 04, 2014 10:40 AM
To: NCNPR
Subject: RE: Link to Account Approved

Hi Jennifer,

I am having trouble accessing the Fedex account I created so can you please create labels for the shipment pick-up? If you can send them to me through e-mail I can print them.

I have seven boxes and the total weight is about 100 lbs. Please schedule the pick up before 3.00 pm today.

Pick up locations is

Rahul Pawar

CFSAN- FDA
5100 Paint Branch Parkway, College Park , MD 20740

Thanks
Rahul

From: NCNPR [<mailto:ncnpr@olemiss.edu>]
Sent: Friday, October 31, 2014 3:25 PM
To: Pawar, Rahul
Subject: RE: Link to Account Approved

I figured it out, thanks.
Jennifer

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Friday, October 31, 2014 2:24 PM
To: NCNPR
Subject: RE: Link to Account Approved

Definitely it is of concern. Let me know if I can be of any help
Have a great weekend.

From: NCNPR [<mailto:ncnpr@olemiss.edu>]
Sent: Friday, October 31, 2014 3:08 PM
To: Pawar, Rahul
Subject: RE: Link to Account Approved

I realize that but we are concerned that access to our account was allowed without our knowledge or approval.

I will call FedEx to see what happened; hopefully our account was not hacked. We just never know!

Have a great day.
Jennifer Michael

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Friday, October 31, 2014 2:02 PM
To: NCNPR
Cc: Ikhlas Khan
Subject: RE: Link to Account Approved

I have used NCNPR account number to schedule Fedex package pick up containing donation items to NCNPR. Jennifer e-mailed me that I will loose the access once the shipment is received by NCNPR.

From: NCNPR [<mailto:ncnpr@olemiss.edu>]
Sent: Friday, October 31, 2014 2:38 PM

To: Pawar, Rahul
Subject: FW: Link to Account Approved

We are interested in finding out how you gained access to our FedEx account without prior approval from our office. See the email we received below. Jennifer Taylor told me she gave you NCNPR's FedEx account number for the shipments.

We are just concerned if you were able to gain access to our FedEx Account without approval from our office administrator who else can do this.

Jennifer W. Michael, Staff Assistant
National Center for Natural Products Research
University of Mississippi
1558 University Circle
University, MS 38677
Phone: 662-915-1005
Fax: 662-915-1006

From: FedEx.com Online Services [<mailto:onlineservices@fedex.com>]
Sent: Friday, October 31, 2014 9:41 AM
To: NCNPR
Subject: Link to Account Approved

The screenshot shows an email interface with a purple header bar. The title 'Link to Account Approved' is centered in the header. Below the header is a navigation bar with links: [fedex.com](#), [Ship](#), [Welcome Center](#), and [Promotions](#). The main body of the email is white and contains the following text:

Dear LARRY WALKER:

Rahul Pawar now has access to using a FedEx account for which you are account administrator.

If you believe this is in error, please [click here](#) to remove the user access. The link provided is active for only 90 days. Once the expiration date has been reached, to remove the user access from your account, please call 1.800.463.3339. Rahul Pawar's email address is

On the right side of the email body, there is a purple sidebar with a white background. It contains the text: 'Special offers -- all in one place' and 'Stay in the know about all the exciting new FedEx promotions and offers that can make your life easier.' At the bottom of the sidebar is a link: [Click here](#) to go to the

rahul.pawar@fda.hhs.gov.

Thank you for choosing FedEx.

Promotions Center.

[Sign up](#) for FedEx eNews to get
Updates about FedEx shipping
services and easy online tools.

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do not reply to this message.

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From: [Pawar, Rahul](#)
To: [BHARATHI AVULA](#)
Cc: [YANHONG WANG](#)
Subject: RE: list of products
Date: Monday, September 14, 2015 12:02:00 PM

Hello, which ones and how much of each do you need? I hope to send by early next week. Thanks.

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Monday, September 14, 2015 11:37 AM
To: Pawar, Rahul
Cc: YANHONG WANG
Subject: RE: list of products

Dear Rahul,

when are U planning to send the listed products???

thanks
bharathi

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Monday, September 14, 2015 7:55 AM
To: BHARATHI AVULA <bavula@olemiss.edu>
Subject: RE: list of products

Thanks!

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Monday, September 14, 2015 8:42 AM
To: Pawar, Rahul
Subject: list of products

could U please email me the products list

thanks
bharathi

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: RE: manuscript title
Date: Friday, April 5, 2013 8:21:22 AM

Thank you Rahul...

This year parents do not have plans to come.

Bharathi

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Friday, April 05, 2013 7:19 AM
To: BHARATHI AVULA
Subject: RE: manuscript title

Have a great time during the meeting. we will be missing it again.
How is uncle aunty, are they travelling to US?

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Friday, April 05, 2013 8:13 AM
To: Pawar, Rahul
Subject: manuscript title

Please see below:

Profiling primaquine metabolites in primary human hepatocytes using UHPLC-QTOF-MS with ¹³C stable isotope labeling. Avula, Bharathi; Tekwani, Babu L.; Chaurasiya, Narayan D.; Nanayakkara, Np dhammika; Wang, Yan-Hong; Khan, Shabana I.; Adelli, Vijender R.; Sahu, Rajnish; Elsohly, Mahmoud A.; McChesney, James D.; et al. Journal of Mass Spectrometry (2013), 48(2), 276-285.

Even I am not planning to go for ASP meeting. This time also Vaishali is coming to ICSB conference. Markus also will be here.

Thank you for your help

Bharathi

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Re: meeting
Date: Friday, April 8, 2011 4:48:13 PM

Good. We gave up on most of them

Sent from my iPhone

On Apr 8, 2011, at 3:56 PM, "Pawar, Rahul" <Rahul.Pawar@fda.hhs.gov> wrote:

Hello Dr. Khan,
I hope you are doing well.
I just thought of updating you that myself and (b) (6) WILL BE attending the meeting.
Looks like many of the FDA people will not be able to attend the meeting because of the budget/shutdown issue.
We will be in oxford Sunday evening and let me know if I can be of any help. See you soon
Best regards
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 301-436-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: [BHARATHI AVULA](#)
Subject: RE: Mitragyna javanica
Date: Thursday, October 16, 2014 10:26:00 AM

Oh! Thanks for your efforts.

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Thursday, October 16, 2014 10:18 AM
To: Pawar, Rahul
Cc: Ikhlas Khan; ZULFIQAR ALI
Subject: FW: Mitragyna javanica

We do not have M. javanica and Vijay tried but could not get

thanks
bharathi

From: VIJAYASANKAR RAMAN
Sent: Thursday, October 16, 2014 8:58 AM
To: BHARATHI AVULA
Subject: RE: Mitragyna javanica

Bharathi,

We don't have M. javanica.

Vijayasankar Raman, Ph.D.
Systematic Botanist
National Center for Natural Products Research
Room # 3027, Thad Cochran Research Center
School of Pharmacy
University of Mississippi
University, MS-38677
Ph. (662) 915-1018

From: BHARATHI AVULA
Sent: Thursday, October 16, 2014 7:57 AM
To: VIJAYASANKAR RAMAN
Cc: Shobhan Singh (ssingh2@go.olemiss.edu)
Subject: Mitragyna javanica

Dear Vijay,

Could you please check and let me know if we have samples of Mitragyna javanica

Thanks
bharathi

From: Ikhlas Khan
Sent: Thursday, October 16, 2014 7:54 AM
To: BHARATHI AVULA; Pawar, Rahul (Rahul.Pawar@fda.hhs.gov)
Cc: ZULFIQAR ALI
Subject: Re: Kratom

We need to get the sample to confirm it.

ik

From: Bharathi Avula <bavula@olemiss.edu>

Date: Thursday, October 16, 2014 at 7:01 AM

To: Rahul Pawar <Rahul.Pawar@fda.hhs.gov>

Cc: Ikhlas Khan <ikhlan@olemiss.edu>, ZULFIQAR ALI <zulfiqar@olemiss.edu>

Subject: FW: Kratom

Dear Rahul,

From literature search, the *M. javanica* did not contain mitragynine (C₂₃H₃₀N₂O₄; 398.2205) but contains javaphylline (C₂₂H₂₆N₂O₅; 398.1841), mitrajavine...

Thank you

bharathi

From: Ikhlas Khan

Sent: Thursday, October 16, 2014 3:55 AM

To: BHARATHI AVULA; ZULFIQAR ALI

Subject: Fwd: Kratom

Can you respond to rahul

Sent from my iPhone

Begin forwarded message:

From: "Pawar, Rahul" <Rahul.Pawar@fda.hhs.gov>

Date: October 16, 2014 at 4:30:52 AM GMT+8

To: "ikhlan@olemiss.edu" <ikhlan@olemiss.edu>

Subject: Kratom

Hello Sir,

One quick question. Does Mitragyna javanica contain Mitragynine?

Thanks

Rahul

Rahul Pawar Ph.D.

Division of Bioanalytical Chemistry

CFSAN-FDA

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 240-402-1795

Fax: 301-436-2622

From: Pawar, Rahul
To: "Premalatha Balachandran"
Subject: RE: Monosaccharides kit
Date: Monday, March 15, 2010 1:15:37 PM

Hi Premalatha,

Sorry for the very late response. I was out of office for sometime and then your email just got buried in pile of junk emails.

I believe that I handed over the sugars to Nirmal before I left. And I am sure it was not in my possession when I left.

(b) (6) is doing well. She talks well (too much!) and spends time happily in her daycare.

How are thick and thin doing! When will (b) (6) go to school?

We are excited to meet you all next month in oxford.

Best regards

Rahul

-----Original Message-----

From: Premalatha Balachandran [<mailto:prembala@olemiss.edu>]

Sent: Tuesday, February 23, 2010 11:14 AM

To: Pawar, Rahul

Subject: Monosaccharides kit

Hi Rahul,

Hope you, (b) (6) are doing fine and we would love to see new (b) (6) pictures. I am sure she should have grown up by now.

Nirmal mentioned to me that you had some monosaccharides kit before.

We just wanted to know to whom you have handed it over?

Thanks,

Premalatha

--

Premalatha Balachandran, Ph.D.,
National Center for Natural Products Research,
Research Institute of Pharmaceutical Sciences, School of Pharmacy,
University of Mississippi, MS-38677, USA.
Ph.662-915-3463
Fax:662-915-7062
Visit me at : <http://home.olemiss.edu/~prembala>

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From: BHARATHI AVULA
To: [Pawar, Rahul](#); vaishjoshi@gmail.com
Subject: RE: News about Valli
Date: Thursday, August 23, 2012 8:17:45 AM

I am sorry to write that Valli passed away....

Bharathi

From: NCNPR
Sent: Wednesday, August 22, 2012 3:17 PM
Subject: News about Valli

To: NCNPR Faculty & Staff, USDA and School of Pharmacy

Dear NCNPR faculty and staff,

This afternoon, we learned that Rangavalli "Valli" Manyam has passed away. When she did not show up for her dialysis appointment, the clinic contacted Annette, and Bharathi went to check and found her body at her apartment. Though not entirely unexpected, I know all of us are saddened, and we remember her many contributions to our family here. So many of you have helped her unselfishly in the last couple of years, and I want to thank all of you for your compassion and care for her.

As the final arrangements are available, we will try to forward that information.

Larry Walker, Director

National Center for Natural Products Research
Thad Cochran Research Center, Room 1019
University of Mississippi
P. O. Box 1848
University, MS 38677
Phone: 662-915-1005
Fax: 662-915-1006

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Re: news
Date: Monday, March 26, 2012 10:43:38 PM

Hi Rahul

Yes (b) (6) is interested but he will confirm after a week or so. I will contact you as soon as I get an answer.

Thanks

IK

From: "Pawar, Rahul" <Rahul.Pawar@fda.hhs.gov>
Date: Mon, 26 Mar 2012 22:41:05 -0400
To: Ikhlas Khan <ikhan@olemiss.edu>
Subject: RE: news

Hello Dr Khan,

Did (b) (6) express interest about the possibility of working at FDA?

I hope preparations for the conference are in full swing. Please let me know if I can be of any help.

Best regards

Rahul

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Wednesday, March 21, 2012 8:28 PM
To: Pawar, Rahul
Subject: Re: news

Hi Rahul

Hope you are doing fine. I talked with Jeanne and she told me that she won't be coming to Oxford. I hope you are coming.

(b) (6) is finishing his 2nd year and I am not sure what he will do in summer but I want to check if there is any possibility at FDA or other places for summer.

IK

From: "Pawar, Rahul" <Rahul.Pawar@fda.hhs.gov>
Date: Mon, 5 Mar 2012 16:55:08 -0500
To: Ikhlas Khan <ikhan@olemiss.edu>
Subject: RE: news

Happy Journey!

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Monday, March 05, 2012 4:53 PM
To: Pawar, Rahul
Subject: Re: news

Everything is fine. Thanks, yes I am going to India tomorrow and will be back on 15th.

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>

Date: Mon, 5 Mar 2012 16:48:22 -0500

To: Ikhlas Khan <ikhan@olemiss.edu>

Subject: RE: news

Great News Sir, Congratulations! Will you be going to India to receive the degree?

I hope all is well in (b) (6) after the tornado forecasts.

Regards to all

Rahul

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]

Sent: Monday, March 05, 2012 10:18 AM

To: Fabricant, Daniel; Calvey, Elizabeth M; Rader, Jeanne I; Dou, Jinhui

Subject: news

Hi

I would to share the news with you. I will be awarded Honorary degree (Honoris Causa) D.Sc from University of Hamdard, Delhi, India this weekend. I would like to thank for your support.

Thanks

IK

From: [Pawar, Rahul](#)
To: [Ikhlas Khan](#)
Subject: RE: next week
Date: Monday, September 22, 2014 3:37:00 PM

Ok, I will join.

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Monday, September 22, 2014 11:08 AM
To: Pawar, Rahul
Subject: Re: next week

Monday Night all COE members will go for dinner together
IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Mon, 22 Sep 2014 15:06:04 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: next week

Good, thanks.

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Monday, September 22, 2014 11:04 AM
To: Pawar, Rahul
Subject: Re: next week

Monday night, it will be a group dinner.
IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Mon, 22 Sep 2014 14:49:50 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: next week

Good, If you have time we can plan something for Sunday or Monday night then. Thanks
Rahul

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Monday, September 22, 2014 10:04 AM
To: Pawar, Rahul
Subject: Re: next week

Thanks, I was thinking to contact you. Yes we are arriving on Sunday afternoon and leave on Tuesday afternoon. Amar and I will come Sunday afternoon and Larry will come later on Sunday. We are staying at Hampton Inn on Greenbelt.

Hope to see you soon.
IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>

Date: Mon, 22 Sep 2014 13:57:21 +0000

To: Ikhlas Khan <ikhlan@olemiss.edu>

Subject: next week

Hello Sir,

How are you doing?

Are you coming to CFSAN next week? Hope to see you

Thanks

Rahul

Rahul Pawar Ph.D.

Division of Bioanalytical Chemistry

CFSAN-FDA

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 240-402-1795

Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: [BHARATHI AVULA](#)
Subject: RE: one clarification
Date: Wednesday, April 30, 2014 9:23:00 AM

Yes, you can do that too. In my case I sonicated the ACN to dissolve the amm-ace (it takes about 1 hr). What analysis are you doing?

I called you some time back. I am coming to Memphis next week and will come to NCNPR for a day tour for the GMP course. We will surely meet but my be for a short period.

Hope to see you, TC

Rahul

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Wednesday, April 30, 2014 8:35 AM
To: Pawar, Rahul
Subject: one clarification

In your Acacia manuscript the mobile phase you used is Ammonium acetate 10 mM (A) and Ammonium acetate in acetonitrile (B)...

Ammonium acetate is not completely soluble in acetonitrile, did you dissolve in water prior to adding acetonitrile??

Thank you

bharathi

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: RE: one clarification
Date: Wednesday, April 30, 2014 9:29:32 AM

I am in other room...

Thank you
bharathi

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Wednesday, April 30, 2014 8:23 AM
To: BHARATHI AVULA
Subject: RE: one clarification

Yes, you can do that too. In my case I sonicated the ACN to dissolve the amm-ace (it takes about 1 hr). What analysis are you doing?

I called you some time back. I am coming to Memphis next week and will come to NCNPR for a day tour for the GMP course. We will surely meet but may be for a short period.

Hope to see you, TC

Rahul

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Wednesday, April 30, 2014 8:35 AM
To: Pawar, Rahul
Subject: one clarification

In your Acacia manuscript the mobile phase you used is Ammonium acetate 10 mM (A) and Ammonium acetate in acetonitrile (B)...

Ammonium acetate is not completely soluble in acetonitrile, did you dissolve in water prior to adding acetonitrile??

Thank you
bharathi

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Re: ORS Dietary Supplement Research Area Coordinator
Date: Thursday, April 16, 2015 4:37:06 PM

Happy to support. We will talk more

Sent from my iPhone

On Apr 16, 2015, at 3:35 PM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov> wrote:

Thank you for all your support!

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Thursday, April 16, 2015 4:28 PM
To: Pawar, Rahul
Subject: Re: ORS Dietary Supplement Research Area Coordinator

Congratulations. Very happy and proud of you.
Wish you all the best

Sent from my iPhone

On Apr 16, 2015, at 1:53 PM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov> wrote:

Just to let you know.

From: Noonan, Gregory
Sent: Wednesday, April 15, 2015 10:33 AM
Subject: ORS Dietary Supplement Research Area Coordinator

I am writing to announce that Dr. Rahul Pawar has agreed to begin serving as the Research Area Coordinator for Dietary Supplements. Dr. Pawar has been with ORS since 2007, first as a visiting scientist and then as a Staff Fellow since 2011. Prior to joining ORS he acquired extensive training and experience in Medicinal and Natural Products, with research focused on isolating and identifying components from various botanicals and the development of analytical methods for their quantification. Since joining FDA he has worked on a number of high profile projects, including identifying adulteration/substitution in dietary supplements containing Muira Puama, characterization of authentic *Acacia rigidula* and finding of adulteration in *Acacia*-containing dietary supplements.

Dr. Pawar has been directly or indirectly involved in nearly all of the dietary supplement projects within the Division of Bioanalytical Chemistry over the past few years and has been a subject matter expert in consultation with the Division of Dietary Supplement

Safety. With supplement related projects now beginning in other Branches and Divisions, this is a perfect time to formalize Dr. Pawar's role. I am confident that Dr. Pawar's knowledge about supplements will be a benefit to addressing technical questions on number of projects. Please give Dr. Pawar your full support as he takes on these new responsibilities. Also, if anyone has any questions or concerns, please feel free to contact me.

Thanks, and Congratulations to Rahul!
Greg

Gregory O. Noonan, PhD
Director, Division of Bioanalytical Chemistry
Food and Drug Administration
5100 Paint Branch Parkway, HFS 706
College Park, MD 20740

PH: 240-402-2250
FAX: 301-436-2634
BB: 240-506-7360
Gregory.Noonan@fda.hhs.gov

From: [Pawar, Rahul](#)
To: ["BHARATHI AVULA"](#)
Subject: RE: paper
Date: Monday, June 8, 2015 3:04:00 PM
Attachments: [s14.pdf](#)

[Here you go..](#)

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Monday, June 08, 2015 2:41 PM
To: Pawar, Rahul
Subject: paper

could U please mail me following paper:

Bharathi Avula, Yan-Hong Wang, Ikhlas A. Khan. Arsenic speciation and fucoxanthin analysis from seaweed dietary supplements using LC-MS. Journal of AOAC International (2015), 98(2), 321-329.

thanks
bharathi

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: RE: paper
Date: Monday, June 8, 2015 3:06:04 PM

thanks

bharathi

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Monday, June 08, 2015 2:04 PM
To: BHARATHI AVULA
Subject: RE: paper

[Here you go..](#)

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Monday, June 08, 2015 2:41 PM
To: Pawar, Rahul
Subject: paper

could U please mail me following paper:

Bharathi Avula, Yan-Hong Wang, Ikhlas A. Khan. Arsenic speciation and fucoxanthin analysis from seaweed dietary supplements using LC-MS. Journal of AOAC International (2015), 98(2), 321-329.

thanks
bharathi

From: bavula@olemiss.edu
To: [""Pawar; Pawar, Rahul](#)
Subject: RE: Paper Request
Date: Thursday, January 28, 2010 3:23:37 PM

Thank you for the paper

Sorry I do not have the list for all the compounds of *Hoodia* but I have the list for our published papers.

If it is not urgent I will try to get from Annette.

Bharathi

From: [Pawar, Rahul](#)
To: ["bavula@olemiss.edu"](mailto:bavula@olemiss.edu)
Subject: RE: Paper Request
Date: Thursday, January 28, 2010 3:16:18 PM

Do you by any chance a list of all the hoodia compounds and their molecular weights?
Don't take extra efforts, if you have it already then send it or just leave it
Thanks
Rahul

From: bavula@olemiss.edu [mailto:bavula@olemiss.edu]
Sent: Thursday, January 28, 2010 2:38 PM
To: ""Pawar; Pawar, Rahul
Subject: Paper Request

Could you please mail me the following paper:



Simultaneous Quantification of Adrenergic Amines and Flavonoids in *C. aurantium*, various *Citrus* species and Dietary Supplements by Liquid Chromatography. *Journal of AOAC International* (2005), 88(6), 1593-1606.

Also could you please also send me the sample letters for EB-1

Thank you
Bharathi

From: [Pawar, Rahul](#)
To: ["BHARATHI AVULA"](#)
Subject: RE: paper request
Date: Friday, August 19, 2011 12:49:00 PM

On line only 2000 onwards issues are available, sorry

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Friday, August 19, 2011 12:35 PM
To: Pawar, Rahul
Subject: paper request

Could you please email me the following paper:

Betz JM, White KD, der Marderosian AH. Gas chromatographic determination of yohimbine in commercial yohimbe products. J AOAC Int. 1995 Sep-Oct;78(5):1189-94.

Thank you
Bharathi

From: [Pawar, Rahul](#)
To: ["bavula@olemiss.edu"](mailto:bavula@olemiss.edu)
Subject: RE: Paper Request
Date: Thursday, January 28, 2010 3:25:08 PM

[Its ok, don't worry, I will make it from the papers](#)

From: bavula@olemiss.edu [mailto:bavula@olemiss.edu]
Sent: Thursday, January 28, 2010 3:24 PM
To: ""Pawar; Pawar, Rahul
Subject: RE: Paper Request

Thank you for the paper

Sorry I do not have the list for all the compounds of *Hoodia* but I have the list for our published papers.

If it is not urgent I will try to get from Annette.

Bharathi

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE: Photo from CFSAN Awards
Date: Monday, September 10, 2012 7:50:57 AM

It was good to see you and congratulations again.

I am happy Bill sent you the pictures. The two person are Dr. Linda Katz, Director, Office of Cosmetics and Colors and Philip C Spiller, Deputy Director for Regulatory Affairs.

Thanks

Rahul

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Saturday, September 08, 2012 8:55 PM
To: Pawar, Rahul
Subject: FW: Photo from CFSAN Awards
Importance: High

Hi Rahul

We arrived safely. I received this photo I know barbara and Mike but don't know other two people in it. Can you give their name and designation.

Thanks

IK

From: bill mindak <bmindak1@yahoo.com>
Reply-To: bill mindak <bmindak1@yahoo.com>
Date: Sat, 8 Sep 2012 08:55:51 -0700
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Photo from CFSAN Awards

Hi Iklas,

Nice to meet you yesterday. Attached is your photo from the CFSAN awards ceremony.

Bill Mindak

From: [Pawar, Rahul](#)
To: ["Aruna Weerasooriya"](#)
Subject: RE: Pictures you asked from Dr. Khan
Date: Wednesday, September 5, 2012 12:51:12 PM

Hi Aruna,
We are doing well and hope you all are too.
Thank you or your help. Do you have any picture for Blue cohosh?
Are you coming to DC? I saw your name on an organizing committee for a meeting.
Thank again and convey our regards to both girls.
Rahul

From: Aruna Weerasooriya [mailto:arunaw@olemiss.edu]
Sent: Wednesday, September 05, 2012 12:18 PM
To: Pawar, Rahul
Subject: Pictures you asked from Dr. Khan

Dear Rahul,
Dr. Khan forwarded me your e-mail and attached are the pictures of what we have at the moment.
Hope all are doing well.
Best,
Aruna

From: [Pawar, Rahul](#)
To: [Ikhlas Khan](#)
Subject: RE: PNAS hoodia article
Date: Thursday, October 9, 2014 8:59:00 AM

I agree and was surprised too. We have published the most comprehensive chemistry on hoodia.
Reviewers need to do better.

This also may be an effort to put life into the Hoodia supplements!

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Wednesday, October 08, 2014 7:26 PM
To: Pawar, Rahul; BHARATHI AVULA
Cc: SHABANA I KHAN
Subject: Re: PNAS hoodia article

No mention of your work. that's the reason Chemistry journal have low impact and we always mention their biological stuff.

IK

From: <Pawar>, Rahul Pawar <Rahul.Pawar@fda.hhs.gov>
Date: Wednesday, October 8, 2014 at 12:26 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>, Bharathi Avula <bavula@olemiss.edu>
Subject: PNAS hoodia article

<http://www.pnas.org/content/111/40/14571.full.pdf+html>

From: [Pawar, Rahul](#)
To: [Brown, Detra](#)
Subject: RE: Postdoc
Date: Friday, July 22, 2016 9:04:00 AM

Detra,
See his email below-

Hi Rahul,
I posted the documents through USPS Priority service and the tracking shows it delivered on 18th July.
Address posted to
Detra Brown
Management Analyst
Food and Drug Administration Center for Food Safety and Applied Nutrition
Office of Regulatory Science
5100 Paint Branch Parkway
College Park, MD 20740

Thanks
Raju

From: Brown, Detra
Sent: Friday, July 22, 2016 7:53 AM
To: Pawar, Rahul
Subject: RE: Postdoc

I have checked, I do not have anything from him in my file or email.

From: Pawar, Rahul
Sent: Friday, July 22, 2016 7:53 AM
To: Brown, Detra
Subject: RE: Postdoc

Thanks Detra,
I am worried. This is what he communicated with me on July 12th "Posted required documents to Detra Brown today."
This means you did not received them? Can you please check?
Thanks
Rahul

From: Brown, Detra
Sent: Friday, July 22, 2016 7:22 AM
To: Pawar, Rahul

Subject: FW: Postdoc

Rahul,

This is the last communication that I have with Dr. Sagi, he never responded to me with the information requested below.

Thanks

*Detra Brown
Management Analyst
Food and Drug Administration
Center for Food Safety
and Applied Nutrition
Office of Regulatory Science
5001 Campus Drive
College Park, MD 20740
240-402-3004 (P)
301-436-2332 (F)*

From: Pawar, Rahul
Sent: Tuesday, June 28, 2016 1:35 PM
To: SATYANARAYANARAJU SAGI (ssagi@olemiss.edu)
Cc: Brown, Detra
Subject: FW: Postdoc

Hi Raju,

The attached ORISE application and eArrive form (Personal, Contact Information, Personal Work Authorization, if applicable, and Federal Work sections) need to be completed and returned with the following documents:

Resume/CV
College Transcripts (Official)
Letters of Reference (2)
Proof of Health Insurance

Send the package to Detra Brown. Let me know if I can assist further.

Detra Brown
Management Analyst
Food and Drug Administration Center for Food Safety and Applied Nutrition
Office of Regulatory Science
5100 Paint Branch Parkway
College Park, MD 20740

240-402-3004 (P)

301-436-2332 (F)

Detra.Brown@fda.hhs.gov

Thanks

Rahul

From: Noonan, Gregory
Sent: Monday, June 27, 2016 3:54 PM
To: Brown, Detra
Cc: Pawar, Rahul
Subject: Postdoc

We have found a person to select for the dietary supplement ORISE project. Can you send Rahul the necessary paperwork so we can get the process underway?

Thanks

Greg

240-701-7415

From: [Pawar, Rahul](#)
To: [SATYANARAYANARAJU SAGI](#)
Subject: RE: Postdoc
Date: Friday, July 22, 2016 7:53:00 AM

Raju, how did you send the documents to Detra?

From: SATYANARAYANARAJU SAGI [mailto:ssagi@olemiss.edu]
Sent: Thursday, July 21, 2016 2:39 PM
To: Pawar, Rahul
Subject: Re: Postdoc

Ok Rahul,

Thanks
Raju

From: Pawar, Rahul <Rahul.Pawar@fda.hhs.gov>
Sent: Thursday, July 21, 2016 1:34:07 PM
To: SATYANARAYANARAJU SAGI
Subject: RE: Postdoc

Not yet..

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Thursday, July 21, 2016 2:30 PM
To: Pawar, Rahul
Subject: Re: Postdoc

Hi Rahul,
Do you hear any updates on my application??

Thanks
Raju

Satyanarayanaraju Sagi
Post Doctoral Research Associate
TCRC Q305
National Center for Natural Products Research
School of Pharmacy
University of Mississippi
Univeristy, MS 38677
Phone: 662 915 7610
Email: ssagi@olemiss.edu

From: Pawar, Rahul <Rahul.Pawar@fda.hhs.gov>

Sent: Tuesday, July 12, 2016 2:12 PM
To: SATYANARAYANARAJU SAGI
Subject: RE: Postdoc

Great!

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Tuesday, July 12, 2016 3:12 PM
To: Pawar, Rahul
Subject: RE: Postdoc

Posted required documents to Detra Brown today.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Wednesday, July 06, 2016 9:18 PM
To: SATYANARAYANARAJU SAGI
Subject: RE: Postdoc

E-arive looks ok, I will probably use only one email address to avoid confusion.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Wednesday, July 06, 2016 3:35 PM
To: Pawar, Rahul
Subject: RE: Postdoc

Hi Rahul,
Please find the attachments of ORISE application and eArrive form.
I filled the details I knew please check these forms and let me know if these are ok.
Dr. Khan is out of office, I will get the reference letter from him and send all the enclosure to Detra Brown.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Tuesday, June 28, 2016 12:35 PM
To: SATYANARAYANARAJU SAGI
Cc: Brown, Detra
Subject: FW: Postdoc

Hi Raju,

The attached ORISE application and eArrive form (Personal, Contact Information, Personal Work Authorization, if applicable, and Federal Work sections) need to be completed and returned with the following documents:

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College Transcripts (Official)
Letters of Reference (2)
Proof of Health Insurance

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Office of Regulatory Science
5100 Paint Branch Parkway
College Park, MD 20740
240-402-3004 (P)
301-436-2332 (F)
Detra.Brown@fda.hhs.gov

Thanks
Rahul

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Sent: Monday, June 27, 2016 3:54 PM
To: Brown, Detra
Cc: Pawar, Rahul
Subject: Postdoc

We have found a person to select for the dietary supplement ORISE project. Can you send Rahul the necessary paperwork so we can get the process underway?

Thanks

Greg
240-701-7415

From: SATYANARAYANARAJU SAGI
To: [Pawar, Rahul](#)
Subject: RE: Postdoc
Date: Tuesday, July 12, 2016 3:12:31 PM

Posted required documents to Detra Brown today.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Wednesday, July 06, 2016 9:18 PM
To: SATYANARAYANARAJU SAGI
Subject: RE: Postdoc

E-arive looks ok, I will probably use only one email address to avoid confusion.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Wednesday, July 06, 2016 3:35 PM
To: Pawar, Rahul
Subject: RE: Postdoc

Hi Rahul,
Please find the attachments of ORISE application and eArrive form.
I filled the details I knew please check these forms and let me know if these are ok.
Dr. Khan is out of office, I will get the reference letter from him and send all the enclosure to Detra Brown.

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Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Tuesday, June 28, 2016 12:35 PM
To: SATYANARAYANARAJU SAGI
Cc: Brown, Detra
Subject: FW: Postdoc

Hi Raju,

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Thanks
Rahul

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Sent: Monday, June 27, 2016 3:54 PM
To: Brown, Detra
Cc: Pawar, Rahul
Subject: Postdoc

We have found a person to select for the dietary supplement ORISE project. Can you send Rahul the necessary paperwork so we can get the process underway?
Thanks

Greg
240-701-7415

From: [Pawar, Rahul](#)
To: [SATYANARAYANARAJU SAGI](#)
Subject: RE: Postdoc
Date: Thursday, July 28, 2016 12:29:00 PM

good

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Thursday, July 28, 2016 12:29 PM
To: Pawar, Rahul
Subject: Re: Postdoc

No.
will send once I get offer letter

Thanks

Satyanarayanaraju Sagi

On Jul 28, 2016, at 11:17 AM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov> wrote:

Raju, have you received an offer letter from ORISE? If or when you have send copy to Detra Brown. Thanks,
Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Thursday, July 28, 2016 10:11 AM
To: Pawar, Rahul
Subject: RE: Postdoc

Hi Rahul,
I emailed Kim regrading my situation. He replied stating that they are going to send me some cards for fingerprints and I give my give my fingerprints in nearby police station.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Monday, July 25, 2016 9:28 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Postdoc

Hi Raju,
Great, things are moving again.
Not official, but your tentative joining date will be end of Sept. This will help you in planning your current work and move.
Best,
Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Monday, July 25, 2016 10:19 AM
To: Pawar, Rahul
Subject: RE: Postdoc

Hi Rahul,

Documents are sent to Detra email on Friday evening.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Friday, July 22, 2016 10:55 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Postdoc

Detra.Brown@fda.hhs.gov

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Friday, July 22, 2016 11:48 AM
To: Pawar, Rahul
Subject: RE: Postdoc

Yes, Can u send Detra email id please.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Friday, July 22, 2016 9:19 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Postdoc

If you have copies of the your package can you scan and send to Detra by email?

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Friday, July 22, 2016 9:02 AM
To: Pawar, Rahul
Subject: RE: Postdoc

Hi Rahul,
I posted the documents through USPS Priority service and the tracking shows it delivered on 18th July.
Address posted to
Detra Brown
Management Analyst

Food and Drug Administration Center for Food Safety and Applied Nutrition
Office of Regulatory Science
5100 Paint Branch Parkway
College Park, MD 20740

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Friday, July 22, 2016 6:53 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Postdoc

Raju, how did you send the documents to Detra?

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Thanks
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Sent: Thursday, July 21, 2016 2:30 PM
To: Pawar, Rahul
Subject: Re: Postdoc

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Do you hear any updates on my application??

Thanks
Raju

Satyanarayanaraju Sagi
Post Doctoral Research Associate

TCRC Q305
National Center for Natural Products Research
School of Pharmacy
University of Mississippi
Univeristy, MS 38677
Phone: 662 915 7610
Email: ssagi@olemiss.edu

From: Pawar, Rahul <Rahul.Pawar@fda.hhs.gov>
Sent: Tuesday, July 12, 2016 2:12 PM
To: SATYANARAYANARAJU SAGI
Subject: RE: Postdoc

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Subject: RE: Postdoc

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Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
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To: SATYANARAYANARAJU SAGI
Subject: RE: Postdoc

E-arive looks ok, I will probably use only one email address to avoid confusion.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Wednesday, July 06, 2016 3:35 PM
To: Pawar, Rahul
Subject: RE: Postdoc

Hi Rahul,
Please find the attachments of ORISE application and eArrive form.
I filled the details I knew please check these forms and let me know if these are ok.
Dr. Khan is out of office, I will get the reference letter from him and send all the enclosure to Detra Brown.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]

Sent: Tuesday, June 28, 2016 12:35 PM
To: SATYANARAYANARAJU SAGI
Cc: Brown, Detra
Subject: FW: Postdoc

Hi Raju,

The attached ORISE application and eArrive form (Personal, Contact Information, Personal Work Authorization, if applicable, and Federal Work sections) need to be completed and returned with the following documents:

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College Park, MD 20740
240-402-3004 (P)
301-436-2332 (F)
Detra.Brown@fda.hhs.gov

Thanks
Rahul

From: Noonan, Gregory
Sent: Monday, June 27, 2016 3:54 PM
To: Brown, Detra
Cc: Pawar, Rahul
Subject: Postdoc

We have found a person to select for the dietary supplement ORISE project. Can you send Rahul the necessary paperwork so we can get the process underway?
Thanks

Greg
240-701-7415

From: Ikhlas A. Khan
To: [Pawar, Rahul](#)
Subject: Re: Postdoc position
Date: Tuesday, October 5, 2010 9:39:30 AM

Hi Rahul

Since we have to open position for postdocs there is no possibility of rushing it. It takes 2-3 months. I am fighting against it. Our labs are also overbooked so advance booking is going on at this point. I will see what I can do.

Any insider news.

IK

At 09:49 AM 10/1/2010, you wrote:

Hello Dr. Khan,

I hope you are doing well.

One of my friend in Memphis is looking for a postdoc position very soon. I have attached is resume for your consideration. He has been working as a pharmacist for few years now after finishing his phd in University of Tennessee in Medi chem.

Please consider his if you have some plans for hiring.

Best regards

Rahul

Ikhlas A. Khan Ph.D.

Assistant Director

Director of FDA Program

Research Professor and

Professor, Department of Pharmacognosy

National Center for Natural Products Research

School of Pharmacy, University of Mississippi

MS 38677

Tel# 662 915 7821

Fax# 662 915 7989

From: [Pawar, Rahul](#)
To: ["BHARATHI AVULA"](#)
Subject: RE: poster photos
Date: Friday, August 12, 2011 9:16:00 AM

Thank you very much, I don't know if you are a bad photographer or the problem is with my face!!! I will send pictures from our camera someee dayyy.
I hope you all are back, How was your vacation? It was great pleasure to meet uncle and aunty, I sure they might have amazed how big (b) (6) has gotten. I wished we could have spent more time with them.
Rest all fine at our end. Take care
And regards to you, uncle and aunty
Rahul, (b) (6)

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Friday, August 12, 2011 9:09 AM
To: Pawar, Rahul
Subject: poster photos

Please find enclosed the poster photos....

I hope all is well....

Good luck
Bharathi

From: [Pawar, Rahul](#)
To: [NCNPR](#)
Subject: RE: PRE-PRINTED FED EX LABELS ATTACHED
Date: Wednesday, November 5, 2014 1:37:00 PM

FYI- Packages picked up today by FedEx.

From: NCNPR [mailto:ncnpr@olemiss.edu]
Sent: Tuesday, November 04, 2014 3:24 PM
To: Pawar, Rahul
Cc: Jennifer S. Taylor
Subject: PRE-PRINTED FED EX LABELS ATTACHED

The 8 separate FedEx labels are attached.

Jennifer W. Michael, Staff Assistant
National Center for Natural Products Research
University of Mississippi
1558 University Circle
University, MS 38677
Phone: 662-915-1005
Fax: 662-915-1006

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Tuesday, November 04, 2014 11:33 AM
To: NCNPR
Subject: RE: Link to Account Approved

240-402-1795

Forgot a big box, there are 8 in total

Approx. weights are

Box 1- 9 lb

Box 2- 15 lb

Box 3- 9 lb

Box 4- 7 lb

Box 5- 10 lb

Box 6- 9 lb

Box 7-40 lb

Box 8- 10 lb

Thanks
Rahul

From: SATYANARAYANARAJU SAGI
To: [Pawar, Rahul](#)
Subject: Re: presentation
Date: Tuesday, June 21, 2016 11:28:00 PM

Satyanarayanaraju Sagi

On Jun 21, 2016, at 11:27 PM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov> wrote:

Send me your slides by email so I can have them ready for presentation.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Tuesday, June 21, 2016 6:56 PM
To: Pawar, Rahul
Subject: Re: Ticket confirmed

Hi Rahul,
Due to bad in DC, I'm have terrible flight journal, two hours flight stayed on Memphis runway. Now it landed in Columbus, Ohio. I will update flight status.

Thanks

Satyanarayanaraju Sagi

On Jun 21, 2016, at 10:04 AM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov> wrote:

Thanks Raju,
Tomorrow when you arrive at the office lobby, after the security check, ask the Security guards to call me (240-402-1795) so I will then escort you in. Do carry your ID as it needed for making the visitors badge.
Check if your hotel provides free shuttle to the College Park metro station. FDA is right in front of the metro station. And do let me know if you need any help.
Best,
Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Monday, June 20, 2016 3:20 PM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

Hi Rahul,

Thanks for the information.
Here is my Bio (made it very simple).

Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Monday, June 20, 2016 1:39 PM
To: SATYANARAYANARAJU SAGI
Subject: RE: Ticket confirmed

Hi Raju,
This is your schedule, if there are any changes I will let you know.
Also, send me your bio for introduction.
Thanks,
Rahul

Schedule for Dr. Sagi

9:00 AM: Dr. Sagi's arrival: Rahul's Office
9: 30-9:45 Meeting with Dr. Cynthia Srigley
9:45-10:15 AM: Lab tour and meeting with other Division members.
10:30 to 11.30 AM: Dr. Sagi's presentation and Q&A
11:45-12:15 PM: Meeting with Dr. Noonan
12:15- 1:30 PM: Lunch
1:30- 2:00 PM: Meeting with Dr. Shaun MacMahon
2:00-2:30 PM: Meeting with Dr. Betsy Yakes
2: 30-3:00: Meeting with Dr. James Wittenberg and Dr. Rakhi Panda
3:00- 3:30: Meeting with Dr. Perry Wang
3:30 PM: Departure

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Monday, June 20, 2016 10:53 AM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

Hi Rahul,

No, I'm Non-vegetarian.
Did u get any agenda? Can you please provide a rough schedule for Wednesday.
I will start around 9:30AM from oxford and reach DC by 4:30PM.
I will call you once I reach my hotel.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Monday, June 20, 2016 9:43 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Ticket confirmed

Raju, are you vegetarian?

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Friday, June 17, 2016 9:03 AM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

OK Rahul

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Thursday, June 16, 2016 11:37 PM
To: SATYANARAYANARAJU SAGI
Subject: RE: Ticket confirmed

Hi Raju, Will let you know next week. Best, Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Thursday, June 16, 2016 5:49 PM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

Hi Rahul,
Just a reminder, have you got any agenda for my interview??

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Thursday, June 09, 2016 10:27 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Ticket confirmed

You are good, job description is pretty much what you do at Umiss. Development and validation of analytical methods. Currently, we have minor interest in natural product isolation but characterization is very relevant. If I get a formal description I may provide. Hope this helps.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Thursday, June 09, 2016 11:22 AM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

Hi Rahul,

If I have job description, I can prepare for interview with group members accordingly.

Thanks

Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]

Sent: Thursday, June 09, 2016 10:09 AM

To: SATYANARAYANARAJU SAGI

Subject: RE: Ticket confirmed

Can you tell me why you need the job description?

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]

Sent: Thursday, June 09, 2016 9:42 AM

To: Pawar, Rahul

Subject: RE: Ticket confirmed

Hi Rahul,

I'm working on presentation. I'm supposed to ask for agenda. If you have some free time and can you send "Job description".

Thanks

Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]

Sent: Wednesday, June 08, 2016 10:12 PM

To: SATYANARAYANARAJU SAGI

Subject: RE: Ticket confirmed

Hi Raju,

That's, good, things are falling in place.

Have you started to prepare a presentation? Be prepared to talk for 30-40 mins and 10-15 mins for questions. I will send final agenda soon.

Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]

Sent: Wednesday, June 08, 2016 5:44 PM

To: Pawar, Rahul

Subject: RE: Ticket confirmed

Hi Rahul,

Hotel reservation confirmed and details are here

Clarion Inn

8601 Baltimore Avenue,

College Park, MD, US, 20740
[+1 \(301\) 474-2800](tel:+13014742800)

One night stay on 21st June (Check in 21st June and Check out 22nd June)

Thanks

Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]

Sent: Tuesday, June 07, 2016 2:10 PM

To: SATYANARAYANARAJU SAGI

Subject: RE: Ticket confirmed

Great, Have you informed Bharathi and Dr. Khan about your interview?

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]

Sent: Tuesday, June 07, 2016 9:41 AM

To: Pawar, Rahul

Subject: Ticket confirmed

Hi Rahul,

Please find the my itinerary.

Will let you know about my lodging once I book my hotel

Thanks

Raju

From: SATYANARAYANARAJU SAGI
To: [Pawar, Rahul](#)
Subject: Re: RE:
Date: Tuesday, May 31, 2016 8:54:07 AM

Sure Rahul

Thanks
Raju

Satyanarayanaraju Sagi

On May 31, 2016, at 7:41 AM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov> wrote:

Raju,
Please keep me posted on your communications with ORISE people.
Thanks,
Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Wednesday, May 25, 2016 9:10 AM
To: Pawar, Rahul
Subject: RE:

I'm sorry forgot to attach the file, here is the file

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Wednesday, May 25, 2016 8:08 AM
To: SATYANARAYANARAJU SAGI
Subject: RE:

To begin I need the file!

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Wednesday, May 25, 2016 9:07 AM
To: Pawar, Rahul
Subject: RE:

Hi Rahul,

Please find the enclosed file. Let me know if you require any information.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]

Sent: Wednesday, May 25, 2016 7:29 AM

To: SATYANARAYANARAJU SAGI

Subject:

Raju,

Can you please fill out this form, as much as you can, and send it back to me.

Roughly, you can reserve time from 9 to 3 pm on 22nd June for the presentation, interview, lunch and meeting here.

You can choose travel to any Washington airports (IAD, DCA, BWI) and then take a shuttle, taxi or metro to reach a hotel in College Park or Beltsville MD area.

Let me know if you need more information.

Thanks,

Rahul

From: SATYANARAYANARAJU SAGI
To: [Pawar, Rahul](#)
Subject: Re: RE:
Date: Monday, June 6, 2016 9:25:29 AM

Okay Rahul
Thank you

Satyanarayanaraju Sagi

On Jun 6, 2016, at 8:15 AM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov> wrote:

Me too. Don't worry, it is their responsibility to arrange for your travel. The interview date should not change.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Monday, June 06, 2016 9:02 AM
To: Pawar, Rahul
Subject: RE:

Hi Rahul,
I didn't hear anything from ORISE people.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Tuesday, May 31, 2016 7:41 AM
To: SATYANARAYANARAJU SAGI
Subject: RE:

Raju,
Please keep me posted on your communications with ORISE people.
Thanks,
Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Wednesday, May 25, 2016 9:10 AM
To: Pawar, Rahul
Subject: RE:

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Thanks
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Subject: RE:

To begin I need the file!

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Wednesday, May 25, 2016 9:07 AM
To: Pawar, Rahul
Subject: RE:

Hi Rahul,

Please find the enclosed file. Let me know if you require any information.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Wednesday, May 25, 2016 7:29 AM
To: SATYANARAYANARAJU SAGI
Subject:

Raju,

Can you please fill out this form, as much as you can, and send it back to me.

Roughly, you can reserve time from 9 to 3 pm on 22nd June for the presentation, interview, lunch and meeting here.

You can choose travel to any Washington airports (IAD, DCA, BWI) and then take a shuttle, taxi or metro to reach a hotel in College Park or Beltsville MD area.

Let me know if you need more information.

Thanks,
Rahul

From: [Pawar, Rahul](#)
To: ["Xiang Fu"](#)
Subject: RE: Regards from Xiang and inquiry about the job opportunity around you
Date: Tuesday, April 24, 2012 2:11:00 PM

Hi Fu Xiang,

Good to hear you are graduating, congratulations.

I will see if any positions are available at FDA, but as of now I have not heard from my boss. What is your visa status?

Have you tried applying at USP, NIST, USDA, NCI and NCCAM? They are one of the few people still working on Natural products. Find e-mail addresses from the ICSB abstract book and send your CV to them. I also saw an advertisement for NP chemist from Pepsi last year, see if they have anything now.

My personal advice to you is to learn LC-MS and HPLC, which is an asset in today world for jobs hunting.

Also, your CV still shows "(expected completion in 2011)," update your CV. You may want to add a section in your CV depicting your expertise.

All the best

Rahul

From: Xiang Fu [mailto:xfu2@olemiss.edu]
Sent: Monday, April 23, 2012 10:31 AM
To: Pawar, Rahul
Subject: Regards from Xiang and inquiry about the job opportunity around you

Dear Rahul,

May I ask you a question about job opportunity in FDA? I am graduating this summer, but I didn't get any offer now. I talked to several people on the ICSB conference this year but it seems funding is a problem for them. So could you please give me some suggestions if you happen to know some openings around you? I attached my CV for your information. Thank you very much!

Sincerely,

Xiang Fu
Graduate Student
Department of Pharmacognosy
School of Pharmacy
The University of Mississippi
University, MS 38677-1848
Phone: 662-915-1033 Fax: 662-915-7989

From: Xiang Fu
To: [Pawar, Rahul](#)
Subject: RE: Regards from Xiang and inquiry about the job opportunity around you
Date: Wednesday, April 25, 2012 11:57:13 AM
Attachments: [fx CV for Olemiss.pdf](#)

Dear Rahul,

Thank you very much for your advice! I updated my CV by changing graduating expectation date and adding the expertise. My visa is F-1, and right now I am student status, but my I-20 form will expire in August this year. I am going to apply for one year OPT (Optimum Performance Training) soon which will allow me to do the training work for one year. I tried USP online application but the documents should go to the human resources and may take longer time. I remember I tried applying the agencies such as USDA or NIH before, but found they need the citizen or permanent resident status for application. I will try to find information again later. I really appreciate your help! Have a nice day!

Sincerely,

Xiang

From: Pawar, Rahul [\[mailto:Rahul.Pawar@fda.hhs.gov\]](mailto:Rahul.Pawar@fda.hhs.gov)
Sent: Tuesday, April 24, 2012 13:11
To: 'Xiang Fu'
Subject: RE: Regards from Xiang and inquiry about the job opportunity around you

Hi Fu Xiang,

Good to hear you are graduating, congratulations.

I will see if any positions are available at FDA, but as of now I have not heard from my boss. What is your visa status?

Have you tried applying at USP, NIST, USDA, NCI and NCCAM? They are one of the few people still working on Natural products. Find e-mail addresses from the ICSB abstract book and send your CV to them. I also saw an advertisement for NP chemist from Pepsi last year, see if they have anything now.

My personal advice to you is to learn LC-MS and HPLC, which is an asset in today world for jobs hunting.

Also, your CV still shows "(expected completion in 2011)," update your CV. You may want to add a section in your CV depicting your expertise.

All the best

Rahul

From: Xiang Fu [\[mailto:xfu2@olemiss.edu\]](mailto:xfu2@olemiss.edu)
Sent: Monday, April 23, 2012 10:31 AM
To: Pawar, Rahul
Subject: Regards from Xiang and inquiry about the job opportunity around you

Dear Rahul,

May I ask you a question about job opportunity in FDA? I am graduating this summer, but I didn't get any offer now. I talked to several people on the ICSB conference this year but it seems funding is a problem for them. So could you please give me some suggestions if you happen to know some openings around you? I attached my CV for your information. Thank you very much!

Sincerely,

Xiang Fu
Graduate Student
Department of Pharmacognosy
School of Pharmacy
The University of Mississippi
University, MS 38677-1848
Phone: 662-915-1033 Fax: 662-915-7989

From: Jennifer S. Taylor
To: [Pawar, Rahul](#)
Subject: Re: Request for a reco letter
Date: Thursday, June 9, 2011 2:16:49 PM

I'm sorry I already put it in the mail.

Jennifer Taylor
Sent from my iPhone

On Jun 9, 2011, at 8:01 AM, "Pawar, Rahul" <Rahul.Pawar@fda.hhs.gov> wrote:

Good morning Jennifer,
If it is possible, can you please also include Dr Walker's recommendation letter to the envelope you were mailing to me today, he provided the scanned copy to my two days back.
Sorry to bother you, thanks
Rahul

.

From: Jennifer S. Taylor [mailto:jnnfrtyl@olemiss.edu]
Sent: Wednesday, June 08, 2011 4:54 PM
To: Pawar, Rahul
Subject: RE: Request for a reco letter

You very welcome and I will send it tomorrow.

Jennifer Taylor

Senior Secretary
National Center for Natural Products Research
P.O. Box 1848
University, MS 38677
Phone (662) 915-1090
Fax (662) 915-7989
[Email \[jnnfrtyl@olemiss.edu\]\(mailto:jnnfrtyl@olemiss.edu\)](mailto:jnnfrtyl@olemiss.edu)

<image001.jpg>

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Wednesday, June 08, 2011 3:47 PM
To: Jennifer S. Taylor
Subject: RE: Request for a reco letter

Hi Jennifer,
How are you doing? Thank you for the letter. Can you also please mail it by regular post at the address below?

Rahul Pawar

5100 Paint Branch Parkway
College Park, MD 20740

Best regards
Rahul

From: Jennifer S. Taylor [mailto:jnnfrtyl@olemiss.edu]
Sent: Wednesday, June 08, 2011 4:43 PM
To: Pawar, Rahul
Subject: FW: Request for a reco letter

Jennifer Taylor

Senior Secretary
National Center for Natural Products Research
P.O. Box 1848
University, MS 38677
Phone (662) 915-1090
Fax (662) 915-7989
Email jnnfrtyl@olemiss.edu

<image001.jpg>

From: Ikhlas A. Khan [mailto:ikhlan@olemiss.edu]
Sent: Friday, June 03, 2011 3:34 PM
To: Jennifer S. Taylor
Subject: Fwd: Request for a reco letter

read and edit it as needed.
IK

X-Default-Received-SPF: pass (skip=forwardok (res=PASS)) x-ip-name=172.16.1.32;
X-SBRS: None
X-MID: 236591182
X-IronPort-Anti-Spam-Filtered: true
X-IronPort-Anti-Spam-Result:
Ap8EACL56E0KuSrB/2dsb2JhbABNBhCCQqReqnufCoMxgnAEn3hR

From: "Pawar, Rahul" <Rahul.Pawar@fda.hhs.gov>
To: "Ikhlas A. Khan" <ikhlan@olemiss.edu>
Date: Fri, 3 Jun 2011 11:13:56 -0400
Subject: Request for a reco letter
Thread-Topic: Request for a reco letter
Thread-Index: AcwiANtnrLG6yQ09T4evGnQQA/baWw==
Accept-Language: en-US

acceptlanguage: en-US

X-Rcpt-To: <ikh@olemiss.edu>

X-LangGuess: English

X-myrbl: Color=White Age=308 Spam=0 Notspam=0

ip=172.16.1.32

X-IP-stats: Incoming Last 0, First 308, in=11644672, out=0, spam=0

ip=172.16.1.32

Hello Sir,

I hope you all are doing well.

I was in need of a favor from you. I was applying for a job position at FDA so I was in need of a recommendation letter from you. I have attached a draft letter, Please feel free to modify it.

This letter can be mailed to me or to Jeanne.

Sorry for the short notice

Best regards

Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 301-436-1795
Fax: 301-436-2622

Content-Type: application/msword; name="Dr Khan reco for Pawar(SF).doc"

Content-Description: Dr Khan reco for Pawar(SF).doc

Content-Disposition: attachment; filename="Dr Khan reco for Pawar(SF).doc";

size=33280; creation-date="Wed, 25 May 2011 14:39:50 GMT";

modification-date="Fri, 03 Jun 2011 13:59:55 GMT"

From: [Pawar, Rahul](#)
To: ["Larry Walker"](#)
Subject: RE: Request for a recommendation letter.
Date: Monday, June 6, 2011 1:11:00 PM

Dear Dr. Walker,
That will be great, I really appreciate your kind help. (b) (6) are doing great; I will convey your regards to them.
Best regards
Rahul

From: Larry Walker [mailto:lwalker@olemiss.edu]
Sent: Monday, June 06, 2011 1:08 PM
To: Pawar, Rahul
Subject: RE: Request for a recommendation letter.

Rahul,
I will try to get this back to Jeanne later today.
Best wishes for this and hello to your family.
Larry

Larry A. Walker, Ph.D.
Director, National Center for Natural Products Research
School of Pharmacy
Associate Director, Basic Sciences (Oxford Campus), Univ. of Mississippi Cancer Institute
University of Mississippi
University, MS 38677 USA
Phone: 662-915-1005
Fax: 662-915-1006

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From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Friday, June 03, 2011 9:39 AM
To: 'Larry Walker'
Subject: Request for a recommendation letter.

Hello Dr. Walker,
I wish you are doing well. It was very saddening to hear about our NCNPR family members suffering losses during the recent tornado incidences in Oxford, but it is comforting to hear everyone was

unharmmed.

I was in need of a favor from you. I was applying for a job position at FDA so I was in need of a recommendation letter from you. I have attached a draft letter, Please feel free to modify it. I have also attached my resume in case you need to refer.

This letter can be mailed to me or to Jeanne.

I hope you can find time in your busy schedule for this kind help.

Best regards

Rahul

Rahul Pawar Ph.D.

Division of Bioanalytical Chemistry

CFSAN-FDA

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 240-402-1795

Fax: 301-436-2622

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Re: Request for a recommendation letter
Date: Wednesday, April 4, 2012 12:44:46 PM

Sure.
ik

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Wed, 4 Apr 2012 12:36:17 -0400
To: Ikhlas Khan <ikh@olemiss.edu>
Subject: Request for a recommendation letter

Dear Dr. Khan,
I hope you all are doing fine.
I need a favor from you. I have to submit application for a peer-review at FDA for promotion to grade GS-14. Can you please provide me a recommendation letter for the same?
A draft is attached, please feel free to edit it.
Thank you for the help
Best regards
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: [Amar Chittiboyina](#)
Subject: RE: Request for chemical
Date: Tuesday, July 19, 2016 10:46:00 PM
Attachments: [image001.png](#)

Hi Amar,

Thanks, good to hear from you. Nice to see you girls during last trip to oxford. (b) (6) has said hi to you. Any plans for DC trip? Btw where is boss travelling?

Yes, 1 mg works for me.

My address is

Center for Food Safety and Applied Nutrition
5100 Paint Branch Parkway
College Park, MD 20740

Best wishes,
Rahul

From: Amar Chittiboyina [mailto:amar@olemiss.edu]
Sent: Tuesday, July 19, 2016 4:34 PM
To: Pawar, Rahul
Subject: RE: Request for chemical

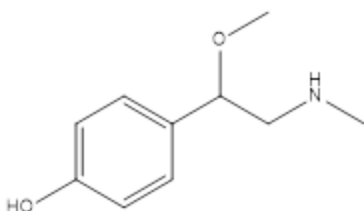
Yes, you are correct. Sampath isolated this compound. At this point, we have only 5mg in our repository. Do you want me to ship around 1 milligram of sample for you?

We are doing well and girls keep me busy. You know it goes. Convey my wishes (b) (6)

Thanks
Amar

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Tuesday, July 19, 2016 2:47 PM
To: Amar Chittiboyina
Subject: RE: Request for chemical

Hi Amar,
How are you?



Chemical Formula: $C_{10}H_{15}NO_2$
Molecular Weight: 181.24

See the NCNPR publication below

Simultaneous Quantification of Adrenergic Amines and Flavonoids in *C. aurantium*, Various *Citrus* Species, and Dietary Supplements by Liquid Chromatography Avula, Bharathi¹; Upparapalli, Sampath Kumar¹; Navarrete, Andres¹; Khan, Ikhlas A.² [Journal of AOAC International](#), Volume 88, Number 6, November 2005, pp. 1593-1606(14)

Thanks,
Rahul

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Tuesday, July 19, 2016 2:29 PM
To: Pawar, Rahul; 'Ikhlas Khan'
Subject: RE: Request for chemical

Dear Rahul,
Can you e-mail me the exact structure of beta-O-Methylsynephrine? Thanks, Amar

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Tuesday, July 19, 2016 7:28 AM
To: Ikhlas Khan
Cc: AMAR GOPAL CHITTIBOYINA
Subject: RE: Request for chemical

Thank you Dr. Khan..

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Monday, July 18, 2016 9:50 PM
To: Pawar, Rahul
Cc: AMAR GOPAL CHITTIBOYINA
Subject: Re: Request for chemical

Amar will check and send if we have it
Ik
Sent from my iPhone

On Jul 18, 2016, at 2:42 PM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov> wrote:

Hello Dr. Khan,
Do you have b-O-methylsynephrine in you laboratory and can you send us 5-10 mg? I remember Sampath kumar worked on it.

Thanks,
Rahul

Rahul Pawar, Ph.D.
Office of Regulatory Science/DBC/BMB
FDA/Center for Food Safety and Applied Nutrition
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795

From: [Pawar, Rahul](#)
To: [Amar Chittiboyina](#)
Subject: RE: Request for chemical
Date: Tuesday, July 26, 2016 11:43:00 AM
Attachments: [image001.png](#)

Hi Amar, got the compound, thanks!

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Monday, July 25, 2016 4:03 PM
To: Pawar, Rahul
Cc: Ikhlas A. Khan
Subject: RE: Request for chemical

Dear Rahul,

Boss went to Copenhagen, Denmark for ASP 2016 meeting. I just shipped the material to by FedEx overnight and the tracking number is 776836152831. Hope the material meets your requirement. Let us know if you need any additional information. Thanks, -Amar

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Tuesday, July 19, 2016 9:47 PM
To: Amar Chittiboyina
Subject: RE: Request for chemical

Hi Amar,

Thanks, good to hear from you. Nice to see you girls during last trip to oxford. (b) (6) has said hi to you. Any plans for DC trip? Btw where is boss travelling?
Yes, 1 mg works for me.

My address is

Center for Food Safety and Applied Nutrition
5100 Paint Branch Parkway
College Park, MD 20740

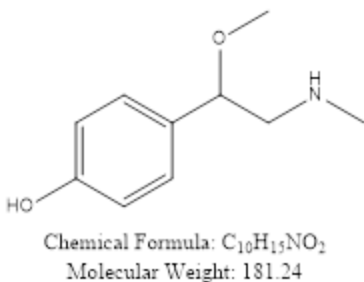
Best wishes,
Rahul

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Tuesday, July 19, 2016 4:34 PM
To: Pawar, Rahul
Subject: RE: Request for chemical

Yes, you are correct. Sampath isolated this compound. At this point, we have only 5mg in our repository. Do you want me to ship around 1 milligram of sample for you?
We are doing well and girls keep me busy. You know it goes. Convey my wishes (b) (6).
Thanks
Amar

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How are you?



See the NCNPR publication below

Simultaneous Quantification of Adrenergic Amines and Flavonoids in *C. aurantium*, Various *Citrus* Species, and Dietary Supplements by Liquid Chromatography Avula, Bharathi¹; Upparapalli, Sampath Kumar¹; Navarrete, Andres¹; Khan, Ikhlas A.² [Journal of AOAC International](#), Volume 88, Number 6, November 2005, pp. 1593-1606(14)

Thanks,
Rahul

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Sent: Tuesday, July 19, 2016 2:29 PM
To: Pawar, Rahul; 'Ikhlas Khan'
Subject: RE: Request for chemical

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Can you e-mail me the exact structure of beta-O-Methylsynephrine? Thanks, Amar

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Sent: Tuesday, July 19, 2016 7:28 AM
To: Ikhlas Khan
Cc: AMAR GOPAL CHITTIBOYINA
Subject: RE: Request for chemical

Thank you Dr. Khan..

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Monday, July 18, 2016 9:50 PM
To: Pawar, Rahul
Cc: AMAR GOPAL CHITTIBOYINA
Subject: Re: Request for chemical

Amar will check and send if we have it

lk

Sent from my iPhone

On Jul 18, 2016, at 2:42 PM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov> wrote:

Hello Dr. Khan,

Do you have b-O-methysynephrine in you laboratory and can you send us 5-10 mg? I remember Sampath kumar worked on it.

Thanks,

Rahul

Rahul Pawar, Ph.D.

Office of Regulatory Science/DBC/BMB

FDA/Center for Food Safety and Applied Nutrition

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 240-402-1795

From: Ikhlas Khan
To: [Pawar, Rahul](#)
Cc: [BHARATHI AVULA](#)
Subject: Re: Request
Date: Tuesday, December 9, 2014 3:03:48 PM

Yes, bharathi will get in touch with you.
Wish you goodluck.
ik

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Fri, 5 Dec 2014 20:30:20 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Request

Hello Dr. Khan,
Hope you are doing well, I just call your office.
Just want to say thank you for the letter and talking to Luke Ackerman for my reviewer for promotion. Should hear about the result in couple of weeks.
I also want to ask you if you could share with us some products you analyzed in your recent ginkgolic acid study? If you can, I will need 5-6 products 10-20 tab/capsules each. We want to use them for performance evaluation purposes.
Will talk to you later, thanks
Best wishes,
Rahul

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Wednesday, November 12, 2014 9:28 AM
To: Pawar, Rahul
Subject: Re: Request

Sure, you will get it today.
ik
ik

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Wed, 12 Nov 2014 14:04:17 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Request

Good morning Dr. Khan,
I have to submit my recommendation letters bit earlier than anticipated. Can you please send me yours by tomorrow? Thank you
Sincerely,
Rahul

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Friday, November 07, 2014 5:15 PM
To: Pawar, Rahul
Subject: Re: Request

Will be happy to do that. I will check on ginkgo paper.

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Fri, 7 Nov 2014 19:13:41 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Request

Hello Dr Khan,

Hope you are doing well.

Need one favor from you. I was apply for a promotion in my office from GS-13 to GS-14 grade.

Please provide me your recommendation letter.

I have attached a draft, please feel free to edit. A pdf copy of the letter is good.

If your ginkgolic acid paper is accepted, can you please send me a copy?

Thank you,

Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: RE: Request
Date: Wednesday, December 10, 2014 9:45:31 AM

The information on products:

15913: Mason Natural G. biloba 500 mg lot # 12795J
15914: Doctors Best Extra Strength Ginkgo lot # 3015A
15918: Life Extension Ginkgo biloba certified extract lot # 107538
15919: MRM Highest Potency Ginkgo B lot # 130226
15921: 21st Century Herbal extract G. biloba extract lot # 5671800
15923: Ginkgold lot # 121006
15925: Ginkgo-24 Source Natural lot # FG-38373

I took picture of all 7 products from phone and mailed you...in case you do not get by evening, let me know

Thanks
bharathi

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Wednesday, December 10, 2014 8:10 AM
To: BHARATHI AVULA
Subject: RE: Request

That will be great, thank you!

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Wednesday, December 10, 2014 9:09 AM
To: Pawar, Rahul
Subject: RE: Request

I will mail you the information of batch # and picture of bottle

Thanks
bharathi

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Wednesday, December 10, 2014 8:02 AM
To: BHARATHI AVULA
Subject: RE: Request

Thank you Bharathi,
That will work. Can you also send the product name and the batch number for those products? We will not share this information anybody outside.

My address is

Rahul Pawar
CFSAN-FDA (HFS-717)
5100 Paint Branch Parkway
College Park, MD
20740

Thank you
Rahul

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Wednesday, December 10, 2014 8:44 AM
To: Pawar, Rahul
Cc: Ikhlas Khan
Subject: RE: Request

Mail me the address to send the products

For one product I can send only about 10 tablets

Thanks
bharathi

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Tuesday, December 09, 2014 3:23 PM
To: BHARATHI AVULA
Subject: FW: Request

Dear Bharathi,

It was good to talk with you after a long time.

I had asked Dr Khan for some dietary supplement samples from your ginkgolic acid study.

I need about 10-20 tablets of the following products (Table 3)

15913

15914

15918

15919

15921

15923

15925

Can you please arrange to ship the samples to me at your earliest.

Thank you for the help

Rahul

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Tuesday, December 09, 2014 3:03 PM
To: Pawar, Rahul
Cc: BHARATHI AVULA
Subject: Re: Request

Yes, bharathi will get in touch with you.
Wish you goodluck.
ik

From: [Pawar, Rahul](#)
To: [Ikhlas Khan](#)
Subject: RE: Request
Date: Tuesday, December 9, 2014 3:24:00 PM

Thanks you Sir!

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Tuesday, December 09, 2014 3:03 PM
To: Pawar, Rahul
Cc: BHARATHI AVULA
Subject: Re: Request

Yes, bharathi will get in touch with you.
Wish you goodluck.
ik

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Fri, 5 Dec 2014 20:30:20 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Request

Hello Dr. Khan,
Hope you are doing well, I just call your office.
Just want to say thank you for the letter and talking to Luke Ackerman for my reviewer for promotion. Should hear about the result in couple of weeks.
I also want to ask you if you could share with us some products you analyzed in your recent ginkgolic acid study? If you can, I will need 5-6 products 10-20 tab/capsules each. We want to use them for performance evaluation purposes.
Will talk to you later, thanks
Best wishes,
Rahul

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Wednesday, November 12, 2014 9:28 AM
To: Pawar, Rahul
Subject: Re: Request

Sure, you will get it today.
ik
ik

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Wed, 12 Nov 2014 14:04:17 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Request

Good morning Dr. Khan,

I have to submit my recommendation letters bit earlier than anticipated. Can you please send me yours by tomorrow? Thank you

Sincerely,

Rahul

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Friday, November 07, 2014 5:15 PM

To: Pawar, Rahul

Subject: Re: Request

Will be happy to do that. I will check on ginkgo paper.

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>

Date: Fri, 7 Nov 2014 19:13:41 +0000

To: Ikhlas Khan <ikhlan@olemiss.edu>

Subject: Request

Hello Dr Khan,

Hope you are doing well.

Need one favor from you. I was apply for a promotion in my office from GS-13 to GS-14 grade.

Please provide me your recommendation letter.

I have attached a draft, please feel free to edit. A pdf copy of the letter is good.

If your ginkgolic acid paper is accepted, can you please send me a copy?

Thank you,

Rahul

Rahul Pawar Ph.D.

Division of Bioanalytical Chemistry

CFSAN-FDA

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 240-402-1795

Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: [Ikhlas Khan](#)
Subject: RE: Request
Date: Wednesday, November 12, 2014 10:48:00 AM

Got the letters, thank you!

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Wednesday, November 12, 2014 9:28 AM
To: Pawar, Rahul
Subject: Re: Request

Sure, you will get it today.

ik
ik

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Wed, 12 Nov 2014 14:04:17 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Request

Good morning Dr. Khan,

I have to submit my recommendation letters bit earlier than anticipated. Can you please send me yours by tomorrow? Thank you

Sincerely,
Rahul

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Friday, November 07, 2014 5:15 PM
To: Pawar, Rahul
Subject: Re: Request

Will be happy to do that. I will check on ginkgo paper.
IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Fri, 7 Nov 2014 19:13:41 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Request

Hello Dr Khan,

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Please provide me your recommendation letter.

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If your ginkgolic acid paper is accepted, can you please send me a copy?

Thank you,
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: ["Jennifer S. Taylor"](#)
Subject: RE: Request
Date: Wednesday, November 12, 2014 10:47:00 AM

Thanks Jennifer!

From: Jennifer S. Taylor [mailto:jnnfrtyl@olemiss.edu]
Sent: Wednesday, November 12, 2014 10:24 AM
To: Pawar, Rahul
Cc: Ikhlas Khan
Subject: RE: Request

Attached please find a recommendation letter from Dr. Ikhlas Khan.

Please let me know if you need anything else.

Jennifer Taylor

Jennifer Taylor
Program Coordinator
University of Mississippi
National Center for Natural Products Research
3012 Thad Cochran
P.O. Box 1848
University, MS 38677

✉ jnnfrtyl@olemiss.edu
☎ 662-915-1090
📠 662-915-7989

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Wednesday, November 12, 2014 8:04 AM
To: Ikhlas Khan
Subject: RE: Request

Good morning Dr. Khan,
I have to submit my recommendation letters bit earlier than anticipated. Can you please send me yours by tomorrow? Thank you
Sincerely,
Rahul

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Friday, November 07, 2014 5:15 PM
To: Pawar, Rahul
Subject: Re: Request

Will be happy to do that. I will check on ginkgo paper.

IK

From: Rahul <Rahul.Pawar@fda.hhs.gov>

Date: Fri, 7 Nov 2014 19:13:41 +0000

To: Ikhlas Khan <ikh@olemiss.edu>

Subject: Request

Hello Dr Khan,

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Thank you,

Rahul

Rahul Pawar Ph.D.

Division of Bioanalytical Chemistry

CFSAN-FDA

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 240-402-1795

Fax: 301-436-2622

From: Krynitsky, Alex
To: [Pawar, Rahul](#)
Subject: RE: reviewer?
Date: Wednesday, October 24, 2012 10:29:00 AM

Hi Rahul,

1. Joe Betz from NIH (don't have his contact off hand).
2. James Harnly USDA-ARS (don't have his contact off hand).

Dr. Rick Myers

Kemin Food Ingredients
2100 Maury Street,
Des Moines
Iowa
50317
United States
Phone: 515.559.5516
Fax: 515.559.5250

Primary E-Mail Address: Rick.Myers@kemin.com

Dr. Bharathi Avula

University of MS.
Phone-662-915-6969

E-mail: bavula@olemiss.edu

From: Pawar, Rahul
Sent: Wednesday, October 24, 2012 10:11 AM
To: Krynitsky, Alex
Subject: reviewer?

Hi Alex,
Can you suggest a potential reviewer for the sweetener review?
Thanks
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: [Krynitsky, Alex](#)
Subject: RE: reviewer?
Date: Wednesday, October 24, 2012 10:29:35 AM

Thanks, I will find it out

From: Krynitsky, Alex
Sent: Wednesday, October 24, 2012 10:29 AM
To: Pawar, Rahul
Subject: RE: reviewer?

Hi Rahul,

1. Joe Betz from NIH (don't have his contact off hand).
2. James Harnly USDA-ARS (don't have his contact off hand).

Dr. Rick Myers

Kemin Food Ingredients
2100 Maury Street,
Des Moines
Iowa
50317
United States
Phone: 515.559.5516
Fax: 515.559.5250

Primary E-Mail Address: Rick.Myers@kemin.com

Dr. Bharathi Avula

University of MS.
Phone-662-915-6969

E-mail: bavula@olemiss.edu

From: Pawar, Rahul
Sent: Wednesday, October 24, 2012 10:11 AM
To: Krynitsky, Alex
Subject: reviewer?

Hi Alex,
Can you suggest a potential reviewer for the sweetener review?
Thanks
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA

5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: Ikhlas Khan
To: [Welch, Cara](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#); [Pawar, Rahul](#)
Subject: Re: rough draft
Date: Thursday, March 2, 2017 10:01:41 AM

Ok, that will help. You can adjust Rahul in your session

Sent from my iPad

On Mar 2, 2017, at 7:18 AM, Welch, Cara <Cara.Welch@fda.hhs.gov> wrote:

Ikhlas and Amar,
Rahul would like to give a presentation – I think his talk can certainly fit into my session on Monday morning, if you think appropriate. Or were you planning on another slot? I didn't see his name in the attached rough draft.

Thanks

Cara

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Wednesday, March 01, 2017 9:53 AM
To: Welch, Cara
Subject: rough draft

Hi Cara

Thanks. Here is the working agenda. There might be a senior guy from Indian government but not sure. Key note address is not a full presentation rather highlight our relationship and talk about CFSAN directions for DS. It can be a short talk (10-15 min)

I appreciate your help

ik

<Agenda blank 2-28-17_v2khan[1].docx>

From: [Welch, Cara](#)
To: [Ikhlas Khan](#)
Cc: [Amar Chittiboyina \(amar@olemiss.edu\)](#); [Pawar, Rahul](#)
Subject: RE: rough draft
Date: Thursday, March 2, 2017 8:18:42 AM
Attachments: [Agenda blank 2-28-17_v2khan\[1\].docx](#)

Ikhlas and Amar,

Rahul would like to give a presentation – I think his talk can certainly fit into my session on Monday morning, if you think appropriate. Or were you planning on another slot? I didn't see his name in the attached rough draft.

Thanks

Cara

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Wednesday, March 01, 2017 9:53 AM
To: Welch, Cara
Subject: rough draft

Hi Cara

Thanks. Here is the working agenda. There might be a senior guy from Indian government but not sure. Key note address is not a full presentation rather highlight our relationship and talk about CFSAN directions for DS. It can be a short talk (10-15 min)

I appreciate your help

ik

From: SHABANA I KHAN
To: [Pawar, Rahul](#)
Subject: Re: Samples for Bioactivity.
Date: Wednesday, February 12, 2014 2:19:37 PM

I received the samples today.

Shabana Khan, Ph.D.
Principal Scientist
Room 2035 NCNPR, School of Pharmacy
University of Mississippi MS 38677
Phone: 662-9151041

From: <Pawar>, Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Monday, February 10, 2014 9:44 AM
To: Shabana Khan <skhan@olemiss.edu>
Subject: Samples for Bioactivity.

Dear Dr. Shabana,

It was nice to talk to you last week.

As discussed I am sending two compounds for biological evaluations to you by UPS. Compound 1 is B26117 (1.9 mg) and compound 2 is B2-10-1-8 (2.5 mg), both will be soluble in DMSO. These are derivatives of a compound named as manicol that was patented by NCI for its anti-HIV activity. Please apply them to suitable assays at your disposal but their cytotoxicity will be essential for submission of our publication. I have told about this to my supervisor and he is ok with this. We appreciate your help in this regard. Hope to talk with you again soon, Thanks

Best regards

Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: [BHARATHI AVULA](#)
Subject: RE: samples
Date: Thursday, December 18, 2014 4:27:00 PM

That explains it! Any plans for holidays?

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Thursday, December 18, 2014 4:27 PM
To: Pawar, Rahul
Cc: REBECCA ANNETTE FORD
Subject: RE: samples

I think it is by normal mail..
It may take some time because of Christmas rush

Bharathi

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Thursday, December 18, 2014 3:22 PM
To: BHARATHI AVULA
Subject: RE: samples

Not received the samples yet. Was it by Fedex , USPS or UPS? Thank you

From: [Pawar, Rahul](#)
To: [BHARATHI AVULA](#)
Subject: RE: samples
Date: Thursday, December 18, 2014 4:21:00 PM

Not received the samples yet. Was it by Fedex , USPS or UPS? Thank you

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Tuesday, December 16, 2014 3:47 PM
To: Pawar, Rahul
Subject: RE: samples

I checked and Annette told me there is no tracking #.
She is on to it

thanks
bharathi

From: Pawar, Rahul [Rahul.Pawar@fda.hhs.gov]
Sent: Tuesday, December 16, 2014 11:24 AM
To: BHARATHI AVULA
Subject: RE: samples

Hi Bharathi,
Can you please track the status of the package or send me the number?
Thanks
Rahul

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Thursday, December 11, 2014 8:02 AM
To: Pawar, Rahul
Cc: Ikhlas Khan
Subject: samples

The samples of Ginkgo products have been dispatched yesterday...

Thanks
bharathi

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: RE: samples
Date: Thursday, December 18, 2014 5:27:55 PM

No plans Rahul

Home Sweet Home

bharathi

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Thursday, December 18, 2014 3:28 PM
To: BHARATHI AVULA
Subject: RE: samples

That explains it! Any plans for holidays?

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Thursday, December 18, 2014 4:27 PM
To: Pawar, Rahul
Cc: REBECCA ANNETTE FORD
Subject: RE: samples

I think it is by normal mail..
It may take some time because of Christmas rush

Bharathi

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Thursday, December 18, 2014 3:22 PM
To: BHARATHI AVULA
Subject: RE: samples

Not received the samples yet. Was it by Fedex , USPS or UPS? Thank you

From: [Pawar, Rahul](#)
To: [SATYANARAYANARAJU SAGI](#)
Subject: RE: Satyanarayanaraju Sagi has shared a file with you using Dropbox
Date: Thursday, June 23, 2016 1:58:00 PM

Raju,
Thanks for coming over for the interview and giving an interesting presentation. We will keep you posted on the developments on our side.
Best,
Rahul

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Thursday, June 23, 2016 1:55 PM
To: Pawar, Rahul
Subject: RE: Satyanarayanaraju Sagi has shared a file with you using Dropbox

Hi Rahul,
Reached oxford last night around 11:30 and resume my duties in NCNPR just now. Nice to see the facility and meet the people at FDA.
Thank you very much for help and support. Let me know if you need information from my side

Thanks

Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Wednesday, June 22, 2016 6:58 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Satyanarayanaraju Sagi has shared a file with you using Dropbox

Try sending it to yourself on your Yahoo or gmail account and then we can open your email here.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Wednesday, June 22, 2016 7:06 AM
To: Pawar, Rahul
Subject: Re: Satyanarayanaraju Sagi has shared a file with you using Dropbox

Hi Rahul,
Landed in DC.. At what time you will be in office..accordingly I will reach there.

Thanks
Raju

Satyanarayanaraju Sagi

On Jun 21, 2016, at 11:30 PM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov> wrote:

You can send it tomorrow, take rest, goodnight.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Tuesday, June 21, 2016 11:29 PM
To: Pawar, Rahul
Subject: Satyanarayanaraju Sagi has shared a file with you using Dropbox

Hi,

Here's a link to "Quality aspects in botanical dietary supplements .pptx" in my Dropbox:

<https://www.dropbox.com/s/xe5fia34z5atssu/Quality%20aspects%20in%20botanical%20dietary%20supplements%20.pptx?dl=0>

Satyanarayanaraju Sagi

From: [Pawar, Rahul](#)
To: [Jennifer S. Taylor](#)
Subject: RE: shipment
Date: Monday, October 6, 2014 1:34:00 PM

Thank you Jennifer! Will let you know when we are ready to ship.

From: Jennifer S. Taylor [mailto:jnnfrtyl@olemiss.edu]
Sent: Monday, October 06, 2014 12:04 PM
To: Pawar, Rahul
Cc: JULIE R MIKELL; Ikhlas Khan; Diana Mobley
Subject: shipment

Please use Fed-Ex number 2118-5351-4 and also send me a copy of the shipping label for my records.

Use the following address:

Julie Mikell
NCNPR
120 Faser
University, MS 38677
662-915-1029

Jennifer Taylor

Jennifer Taylor
Program Coordinator
University of Mississippi
National Center for Natural Products Research
301Q Thad Cochran
P.O. Box 1848
University, MS 38677

✉ jnnfrtyl@olemiss.edu
☎ 662-915-1090
📠 662-915-7989

From: [Pawar, Rahul *](#)
To: ["TROY J SMILLIE"](#)
Subject: RE: shipment
Date: Thursday, September 8, 2011 1:38:00 PM

Troy, Received the package! Thanks
Rahul

From: TROY J SMILLIE [mailto:tsmillie@olemiss.edu]
Sent: Wednesday, September 07, 2011 11:00 AM
To: Pawar, Rahul *; VIJAYASANKAR RAMAN
Subject: shipment

Dear Rahul,

The tracing number for your shipment is 7974 8650 4410 via fed ex. You should receive it tomorrow.

Troy Smillie, Ph.D.
Senior Research Scientist
Thad Cochran National Center for Natural Products Research
School of Pharmacy
University of Mississippi
University, MS 38677
Phone 662-915-1168
Fax 662-915-7062
<http://www.olemiss.edu/depts/pharmacy/php/sopquery3.php?id=69>

From: [Pawar, Rahul *](#)
To: ["TROY J SMILLIE"](#)
Subject: RE: shipment
Date: Wednesday, September 7, 2011 1:59:00 PM

Dear Troy,
Thank you very much, great help. I will let you know when it arrives.
Is boss in turkey, when he will be back?
Rahul

From: TROY J SMILLIE [mailto:tsmillie@olemiss.edu]
Sent: Wednesday, September 07, 2011 11:00 AM
To: Pawar, Rahul *; VIJAYASANKAR RAMAN
Subject: shipment

Dear Rahul,

The tracing number for your shipment is 7974 8650 4410 via fed ex. You should receive it tomorrow.

Troy Smillie, Ph.D.
Senior Research Scientist
Thad Cochran National Center for Natural Products Research
School of Pharmacy
University of Mississippi
University, MS 38677
Phone 662-915-1168
Fax 662-915-7062
<http://www.olemiss.edu/depts/pharmacy/php/sopquery3.php?id=69>

From: JULIE R MIKELL
To: [Pawar, Rahul](#)
Subject: Re: shipment
Date: Tuesday, October 7, 2014 4:29:37 PM

Yes, thank you.

From: <Pawar>, Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tuesday, October 7, 2014 3:28 PM
To: NCNPR NCNPR <rimikell@olemiss.edu>
Subject: RE: shipment

Good, I will send few of different sizes then.

From: JULIE R MIKELL [<mailto:rimikell@olemiss.edu>]
Sent: Tuesday, October 07, 2014 4:27 PM
To: Pawar, Rahul
Subject: Re: shipment

We could always use more. We have an extended collection rack on ours.

Thanks,
Julie

From: <Pawar>, Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Tuesday, October 7, 2014 3:22 PM
To: NCNPR NCNPR <rimikell@olemiss.edu>
Subject: RE: shipment

Hi Julie,
Do you need some isolera racks too? We have many extra and send them with the columns if that helps you. Thanks
Rahul

From: Jennifer S. Taylor [<mailto:jnnfrtyl@olemiss.edu>]
Sent: Monday, October 06, 2014 12:04 PM
To: Pawar, Rahul
Cc: JULIE R MIKELL; Ikhlas Khan; Diana Mobley
Subject: shipment

Please use Fed-Ex number 2118-5351-4 and also send me a copy of the shipping label for my records.

Use the following address:

Julie Mikell
NCNPR
120 Faser

University, MS 38677
662-915-1029

Jennifer Taylor

Jennifer Taylor
Program Coordinator
University of Mississippi
National Center for Natural Products Research
301Q Thad Cochran
P.O. Box 1848
University, MS 38677

✉ jnnfrtyl@olemiss.edu

☎ 662-915-1090

☎ 662-915-7989

From: [Pawar, Rahul](#)
To: icsb@olemiss.edu
Subject: RE: Slides from your ICSB oral presentation
Date: Thursday, April 28, 2016 7:15:00 PM

ICSB Team,
I will prefer not to publish my slides. Thank you.
Rahul

From: icsb@olemiss.edu [mailto:icsb@olemiss.edu]
Sent: Thursday, April 28, 2016 5:09 PM
To: icsb@olemiss.edu
Subject: Slides from your ICSB oral presentation

Greetings from the Oxford ICSB web team.

We would like to ask for your permission to publish your slides on the previous conferences page of our web site at <http://oxfordicsb.org>. The slides will be downloadable in the form of a locked (non-editable) PDF, converted from the Powerpoint file from your presentation.

If you would like to grant us permission, simply reply to this email. If you would like us to delete your slides from our records, please let us know that as well. If you would like to grant permission, but need to edit or remove some slides from the file, feel free to send us an edited PDF or Powerpoint file, or simply let our team know which slides to alter and we will send another email to confirm after edits are made.

Thank you so much for your contribution to the ICSB, and we look forward to hearing from you on this matter!

Regards,

Oxford ICSB Web Team

icsb@olemiss.edu

<http://oxfordicsb.org>

<http://facebook.com/OxfordICSB>

From: [Pawar, Rahul](#)
To: [BHARATHI AVULA](#)
Subject: RE: status of products
Date: Wednesday, May 13, 2015 12:07:00 PM

I will keep you posted on any progress, thanks!

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Wednesday, May 13, 2015 12:06 PM
To: Pawar, Rahul
Subject: RE: status of products

OK....thanks

bharathi

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Wednesday, May 13, 2015 10:40 AM
To: BHARATHI AVULA
Subject: RE: status of products

Ok, we have not bought those supplements yet.

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Wednesday, May 13, 2015 11:39 AM
To: Pawar, Rahul
Subject: RE: status of products

Once upon a time U mailed Dr Khan about the plants to check for analytical methods and those products U people are going to buy...I was checking on those dietary supplements

bharathi

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Wednesday, May 13, 2015 10:36 AM
To: BHARATHI AVULA
Subject: RE: status of products

Hello Bharathi,
I did not understand, which products are you referring to?
Rahul

From: BHARATHI AVULA [<mailto:bavula@olemiss.edu>]
Sent: Tuesday, May 12, 2015 8:37 AM
To: Pawar, Rahul
Subject: status of products

what is the status of commercial products??

thanks

bharathi

From: Oxford ICSB
To: [Pawar, Rahul](#)
Subject: Re: Submission of Poster
Date: Monday, December 6, 2010 3:41:13 PM

Dear Rahul,

Thank you for the abstract. Look forward to seeing you.

Troy

International Conference on the Science of Botanicals
National Center for Natural Products Research
University MS 38677
Phone 662-915-1090
Fax 662-915-7989
Email ICSB@olemiss.edu
www.OxfordICSB.org

From: "Pawar, Rahul" <Rahul.Pawar@fda.hhs.gov>
Date: Thu, 2 Dec 2010 15:54:56 -0500
To: "'ICSB@olemiss.edu'" <ICSB@olemiss.edu>
Conversation: Submission of Poster
Subject: Submission of Poster

Dear Organizers,
Please find my abstract attached for presentation as a poster at the ICSB meeting.
Hope to see you in April
Happy Holidays
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 301-436-1795
Fax: 301-436-2622

From: [Handy, Sara](#)
To: [NATASCHA TECHEN](#)
Subject: RE: summary?
Date: Tuesday, July 26, 2016 8:15:22 AM

Hey Natascha,

I hope you are doing well and that it isn't too hot there (we are around 100 all week and it feels terrible...)

I wanted to touch base with you on this again. The idea of looking into our next gen data further with your nuclear targets is very exciting to me.

Sara

From: NATASCHA TECHEN [mailto:ntechen@olemiss.edu]
Sent: Friday, April 22, 2016 1:30 PM
To: Handy, Sara
Subject: RE: summary?

Hi Sara,
Was a very hectic meeting for me too...
Let me talk to Dr. Khan this afternoon and I'll get back with you after that. Need to discuss with him which way we want to go.

Talk to you soon,
Natascha

"Never, never be afraid to do what's right, especially if the well-being of a person or animal is at stake. Society's punishments are small compared to the wounds we inflict on our soul when we look the other way." Martin Luther King Jr.

Natascha Techen, PhD
Research Scientist- Plant Molecular Biologist
University of Mississippi
National Center for Natural Products Research
P.O. Box 1848
University, MS 38677-1848

Room 2050
Phone (office): 662-915-1090 (Jennifer Taylor)
Cell: (b) (6)
e-mail: ntechen@olemiss.edu

From: Handy, Sara [mailto:Sara.Handy@fda.hhs.gov]

Sent: Tuesday, April 19, 2016 12:51 PM

To: NATASCHA TECHEN <ntechen@olemiss.edu>

Subject: summary?

Hey!!! I am sorry I never got a chance to sit down with you last week. I really enjoyed the conference and will be back again!

Can we put together a one pager for what we are thinking about doing together for me to present to my management? I can start on this from my end but I was curious what all you were thinking about. I think there are some strong places for collaboration!

Sara

Sara M. Handy, Ph.D.

Research Biologist

Office of Regulatory Science/DAC/MDB

FDA/Center for Food Safety and Applied Nutrition

5100 Paint Branch Parkway

College Park, MD 20740

240-402-3063

Sara.Handy@fda.hhs.gov

~The contents of this message are mine personally and do not reflect the position taken by the U.S. government or the FDA~

From: Ikhlas A. Khan
To: [Pawar, Rahul](#)
Subject: Re: Thank you note
Date: Wednesday, June 8, 2011 4:58:57 PM

No problem, Hope it will work. Is this position with Beth.
Did Bhutani got his visa. I will invite him again.
IK

Dear Dr. Khan,

Thank you for the reco letter.

Dr. Bhutani was eager to present a talk in the 2012 conference. Do you have plans for inviting him?

Best regards

Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 301-436-1795
Fax: 301-436-2622

--

From: [Pawar, Rahul](#)
To: ["BHARATHI AVULA"](#)
Subject: RE: Thank You
Date: Monday, July 18, 2011 2:36:00 PM

Congratulations! Long wait but it is worth.
Talk to you soon
Rahul

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Monday, July 18, 2011 12:57 PM
To: YANHONG WANG; Waseem Gul (wgul@elsohly.com); meiwang@olemiss.edu; Jennifer S. Taylor; Annette Ford; Pawar, Rahul
Subject: Thank You

Dear All,

I would like to inform you all that I received (b) (6) and I would like to thank all your support and help...

Thank you all

Bharathi

From: Ikhlas Khan
To: [Li, Jing](#)
Cc: [Pawar, Rahul](#)
Subject: Re: thanks from Jing
Date: Tuesday, August 27, 2013 9:34:50 PM

Dear Jing

I have not heard back from you after ASP. Did any progress been made for USP job? I am here in Washington and discuss with FDA folks for a possibility. They don't have position but postdoc position might be a possibility.

If you are interested please let me know or contact Rahul Pawar
<Rahul.Pawar@fda.hhs.gov>

IK

From: "Li, Jing" <LiJ7@uthscsa.edu>
Date: Wed, 3 Jul 2013 20:47:47 +0000
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: thanks from Jing

Dear Dr. Khan,

I have received your recommendation letter. Thank you so much for your help.
I will be in ASP meeting and look forward to talking with you soon.

Best regards

Jing

From: SATYANARAYANARAJU SAGI
To: [Pawar, Rahul](#)
Subject: Re: Ticket confirmed
Date: Tuesday, June 21, 2016 11:27:48 PM

Ok.. I will update it..

Satyanarayanaraju Sagi

On Jun 21, 2016, at 11:26 PM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov> wrote:

Oh no. hope you can make it. Take a taxi, there should not be very bad traffic on the way out of Dc in the morning.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Tuesday, June 21, 2016 11:25 PM
To: Pawar, Rahul
Subject: Re: Ticket confirmed

No, in Columbus Ohio. Flight cancelled for tonight.
They booked early morning flight at 5:25AM. I will directly come to Ur office around 8:30am.

Good night

Satyanarayanaraju Sagi

On Jun 21, 2016, at 11:18 PM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov> wrote:

I am sorry to hear that. I hope you reach DC safely. Please keep me posted.

From: SATYANARAYANARAJU SAGI [<mailto:ssagi@olemiss.edu>]
Sent: Tuesday, June 21, 2016 6:56 PM
To: Pawar, Rahul
Subject: Re: Ticket confirmed

Hi Rahul,
Due to bad in DC, I'm have terrible flight journal, two hours flight stayed on Memphis runway. Now it landed in Columbus, Ohio. I will update flight status.

Thanks

Satyanarayanaraju Sagi

On Jun 21, 2016, at 10:04 AM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov>

wrote:

Thanks Raju,

Tomorrow when you arrive at the office lobby, after the security check, ask the Security guards to call me (240-402-1795) so I will then escort you in. Do carry your ID as it needed for making the visitors badge.

Check if your hotel provides free shuttle to the College Park metro station. FDA is right in front of the metro station. And do let me know if you need any help.

Best,

Rahul

From: SATYANARAYANARAJU SAGI

[<mailto:ssagi@olemiss.edu>]

Sent: Monday, June 20, 2016 3:20 PM

To: Pawar, Rahul

Subject: RE: Ticket confirmed

Hi Rahul,

Thanks for the information.

Here is my Bio (made it very simple).

Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]

Sent: Monday, June 20, 2016 1:39 PM

To: SATYANARAYANARAJU SAGI

Subject: RE: Ticket confirmed

Hi Raju,

This is your schedule, if there are any changes I will let you know.

Also, send me your bio for introduction.

Thanks,

Rahul

Schedule for Dr. Sagi

9:00 AM: Dr. Sagi's arrival: Rahul's Office

9: 30-9:45 Meeting with Dr. Cynthia Srigley

9:45-10:15 AM: Lab tour and meeting with other Division members.

10:30 to 11.30 AM: Dr. Sagi's presentation and Q&A

11:45-12:15 PM: Meeting with Dr. Noonan
12:15- 1:30 PM: Lunch
1:30- 2:00 PM: Meeting with Dr. Shaun MacMahon
2:00-2:30 PM: Meeting with Dr. Betsy Yakes
2: 30-3:00: Meeting with Dr. James Wittenberg and Dr. Rakhi Panda
3:00- 3:30: Meeting with Dr: Perry Wang
3:30 PM: Departure

From: SATYANARAYANARAJU SAGI
[<mailto:ssagi@olemiss.edu>]
Sent: Monday, June 20, 2016 10:53 AM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

Hi Rahul,

No, I'm Non-vegetarian.
Did u get any agenda? Can you please provide a rough schedule for Wednesday.
I will start around 9:30AM from oxford and reach DC by 4:30PM.
I will call you once I reach my hotel.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Monday, June 20, 2016 9:43 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Ticket confirmed

Raju, are you vegetarian?

From: SATYANARAYANARAJU SAGI
[<mailto:ssagi@olemiss.edu>]
Sent: Friday, June 17, 2016 9:03 AM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

OK Rahul

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]

Sent: Thursday, June 16, 2016 11:37 PM
To: SATYANARAYANARAJU SAGI
Subject: RE: Ticket confirmed

Hi Raju, Will let you know next week. Best, Rahul

From: SATYANARAYANARAJU SAGI
[<mailto:ssagi@olemiss.edu>]
Sent: Thursday, June 16, 2016 5:49 PM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

Hi Rahul,
Just a reminder, have you got any agenda for my interview??

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Thursday, June 09, 2016 10:27 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Ticket confirmed

You are good, job description is pretty much what you do at Umiss. Development and validation of analytical methods. Currently, we have minor interest in natural product isolation but characterization is very relevant. If I get a formal description I may provide. Hope this helps.

From: SATYANARAYANARAJU SAGI
[<mailto:ssagi@olemiss.edu>]
Sent: Thursday, June 09, 2016 11:22 AM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

Hi Rahul,
If I have job description, I can prepare for interview with group members accordingly.

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Thursday, June 09, 2016 10:09 AM
To: SATYANARAYANARAJU SAGI
Subject: RE: Ticket confirmed

Can you tell me why you need the job description?

From: SATYANARAYANARAJU SAGI
[<mailto:ssagi@olemiss.edu>]
Sent: Thursday, June 09, 2016 9:42 AM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

Hi Rahul,
I'm working on presentation. I'm supposed to ask for agenda. If you have some free time and can you send "Job description".

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Wednesday, June 08, 2016 10:12 PM
To: SATYANARAYANARAJU SAGI
Subject: RE: Ticket confirmed

Hi Raju,
That's, good, things are falling in place.
Have you started to prepare a presentation? Be prepared to talk for 30-40 mins and 10-15 mins for questions. I will send final agenda soon.
Rahul

From: SATYANARAYANARAJU SAGI
[<mailto:ssagi@olemiss.edu>]
Sent: Wednesday, June 08, 2016 5:44 PM
To: Pawar, Rahul
Subject: RE: Ticket confirmed

Hi Rahul,

Hotel reservation confirmed and details are here

Clarion Inn
8601 Baltimore Avenue,
College Park, MD, US, 20740
[+1 \(301\) 474-2800](tel:+13014742800)

One night stay on 21st June (Check in 21st June and Check out 22nd June)

Thanks
Raju

From: Pawar, Rahul [<mailto:Rahul.Pawar@fda.hhs.gov>]
Sent: Tuesday, June 07, 2016 2:10 PM
To: SATYANARAYANARAJU SAGI

Subject: RE: Ticket confirmed

Great, Have you informed Bharathi and Dr. Khan about your interview?

From: SATYANARAYANARAJU SAGI
[<mailto:ssagi@olemiss.edu>]
Sent: Tuesday, June 07, 2016 9:41 AM
To: Pawar, Rahul
Subject: Ticket confirmed

Hi Rahul,

Please find the my itinerary.

Will let you know about my lodging once I book my hotel

Thanks

Raju

From: BHARATHI AVULA
To: [Pawar, Rahul](mailto:Pawar,Rahul)
Subject: RE: TOC list- Hope this helps
Date: Monday, June 17, 2013 10:28:38 AM

Yes please....he is back

bharathi

Bharathi Avula, Ph.D.
Senior Research Scientist
Thad Cochran National Center for Natural Products Research
School of Pharmacy
University of Mississippi
University, MS 38677
(662) 915-6969

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Monday, June 17, 2013 8:14 AM
To: BHARATHI AVULA
Subject: RE: TOC list- Hope this helps

Hello, Is boss back?

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Monday, June 17, 2013 8:15 AM
To: Pawar, Rahul
Subject: RE: TOC list- Hope this helps

Thank you Rahul (I found in junk mail)

bharathi

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Thursday, June 13, 2013 8:22 AM
To: BHARATHI AVULA
Subject: TOC list- Hope this helps

From: Ikhlas Khan
To: [Jim Duke](#); [Handy, Sara](#); [Pawar, Rahul](#)
Cc: [Ottesen, Andrea](#)
Subject: Re: U. Miss.
Date: Thursday, October 29, 2015 10:32:01 AM

Dear Jim

I am back in office. Please let me know whom should I talk. I can be reached
At 662 915 7821
Cell (b) (6)
ik

From: Jim Duke <jimduke13@verizon.net>
Date: Monday, October 26, 2015 at 1:48 PM
To: "Handy, Sara" <Sara.Handy@fda.hhs.gov>, Rahul <Rahul.Pawar@fda.hhs.gov>
Cc: "Ottesen, Andrea" <Andrea.Ottesen@fda.hhs.gov>, Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Re: U. Miss.

Actually, that sounds like the best option by far so far. May I pass this on to the decision man, Dr. Gary Kinard, USDA.. He might wish to talk to Dr.Khan when he is here Thurs., I'm sure Dr Khan will be short of time. But bring him out to see our garden if possible

From: [Jim Duke](#)
Sent: Monday, October 26, 2015 12:48 PM
To: [Handy, Sara](#) ; [Pawar, Rahul](#)
Cc: [Ottesen, Andrea](#)
Subject: U. Miss.

From: [Handy, Sara](#)
Sent: Monday, October 26, 2015 12:35 PM
To: [Jim Duke](#) ; [Pawar, Rahul](#)
Cc: [Ottesen, Andrea](#)
Subject: RE: plant collected

Hi Jim,

We work very closely with University of Mississippi which is a Center of Excellence for FDA (<http://pharmacy.olemiss.edu/ncnpr/fda-partnership/>). They also have a medicinal plant garden: <http://pharmacy.olemiss.edu/ncnpr/the-maynard-w-quimby-medicinal-plant-garden/present-day-garden/>. Dr. Pawar spoke to the head of the dietary supplements group there and they are very interested in hosting the site for you. Apparently the head- Iklas Khan, will be here on Thursday and I will speak with him about it then. Perhaps he can get in touch with you after that?

Let me know if this option interests you!

Sincerely,

Sara Handy

From: Jim Duke [mailto:(b) (6) .net]
Sent: Monday, October 26, 2015 11:23 AM
To: Handy, Sara; Ottesen, Andrea
Cc: Zhang, Ning; Ramachandran, Padmini *; Helen Metzman
Subject: Re: plant collected

Thanks Sara. You all were very busy,

I changed the spelling of japonicus and Hydrastis. The USDA nomenclature does not list Illicium parviflora, Perhaps it was a slip of the pen?

James A. (Jim) Duke

(b) (6)

(b) (6) .net

From: Handy, Sara
Sent: Monday, October 26, 2015 10:52 AM
To: Ottesen, Andrea ; (b) (6) .net
Cc: Zhang, Ning ; Ramachandran, Padmini *
Subject: RE: plant collected

Hi Jim,

Here is a complete list of what we collected:

Mitragyna speciosa
Petasites japonicus
Valeriana officianalis
Passiflora incarnata
Artemisia annua
Scutellaria lateriflora
Scutellaria baicalensis
Scutellaria barbata
Actaea racemosa
Hydrastis canadensis
Ephedra sinica
Illicium parviflora
Hypericum perforatum
Hypericum punctatum
Eleutherococcus senticosus

(Let me know if you see a spelling error).

Ning has saved a portion in silica and a larger portion to do both general DNA extraction and some chloroplast enrichment work all with the goal to sequence the entire chloroplast genome with each plant.

I am also speaking with the head of our dietary supplement working group, Rahul Pawar, to see if we might be able to host your database. It turns out he was very sad he didn't get a chance to come to the farm- he has known about it for a long time but never been. He is a pharmacist by training but has worked on botanical dietary supplements on the chemistry side for a very long time.

Ssara

From: Ottesen, Andrea
Sent: Monday, October 26, 2015 10:46 AM
To: j(b) (6) ^{(b) (6)} [.net](#)
Cc: Zhang, Ning; Handy, Sara; Ramachandran, Padmini *
Subject: plant collected

Hi Jim,

We took some:

Artemesia annua,
Hypericum punctatum and hopefully perforatum,
Black cohosh,
Passiflora incarnata,
Skullcap – at Helen's request
Valerian
Mitragyana sp?
Ephedra,
Siberian ginseng

Probably a couple other things too...

I will let Ning and or Sara update you with the precise list – of what they move forward with –
Some items might go to facilitating the streamlining of the methods!

ao

Andrea Ottesen PhD
Research Area Coordinator for Metagenomics

Molecular Methods and Subtyping Branch
Division of Microbiology, ORS CFSAN FDA
Andrea.Ottesen@fda.hhs.gov
240 402 3043

From: [Pawar, Rahul](#)
To: icsb@olemiss.edu
Subject: RE: Upcoming ICSB-ASP
Date: Monday, March 21, 2016 3:23:00 PM
Attachments: [Rahul Pawar-Bio. docx.docx](#)

Hi Jennifer,

Please find attached my Bio for the abstract book.

I will update on my travel plans after I reserve the flight tickets. Currently, I have made a reservation at Hampton Inn near the Conference center for my stay.

Correction: "and" can be taken out between CFSAN and FDA for my affiliation in the agenda.

Thanks you!

Rahul

From: icsb@olemiss.edu [mailto:icsb@olemiss.edu]
Sent: Monday, March 21, 2016 1:03 PM
To: Pawar, Rahul
Subject: Upcoming ICSB-ASP

I just wanted to touch base with you about our upcoming ICSB-ASP meeting. Please find our tentative agenda attached. Would you please review and see if all of your information is correct before we send it to the printer. It would be great if you would send me copy of your itinerary and you hotel information? Will you need the shuttle service? I am listing my contact information below in case you need anything or have any questions. I will be available most anytime.

Jennifer Taylor
662-915-1090 OFFICE
(b) (6) CELL

International Conference on the Science of Botanicals
National Center for Natural Products Research
University MS 38677
Phone 662-915-1090
Fax 662-915-7989
Email ICSB@olemiss.edu
www.OxfordICSB.org

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replying to this e-mail.

From: icsb@olemiss.edu
To: [Pawar, Rahul](#)
Subject: RE: Upcoming ICSB-ASP
Date: Monday, March 21, 2016 4:00:46 PM

Great thanks. I have made the corrections.

International Conference on the Science of Botanicals
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From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Monday, March 21, 2016 2:24 PM
To: icsb@olemiss.edu
Subject: RE: Upcoming ICSB-ASP

Hi Jennifer,
Please find attached my Bio for the abstract book.
I will update on my travel plans after I reserve the flight tickets. Currently, I have made a reservation at Hampton Inn near the Conference center for my stay.
Correction: "and" can be taken out between CFSAN and FDA for my affiliation in the agenda.
Thanks you!
Rahul

From: icsb@olemiss.edu [mailto:icsb@olemiss.edu]
Sent: Monday, March 21, 2016 1:03 PM
To: Pawar, Rahul
Subject: Upcoming ICSB-ASP

I just wanted to touch base with you about our upcoming ICSB-ASP meeting. Please find our tentative agenda attached. Would you please review and see if all of your information is correct before we send it to the printer. It would be great if you would send me copy of your

itinerary and you hotel information? Will you need the shuttle service? I am listing my contact information below in case you need anything or have any questions. I will be available most anytime.

Jennifer Taylor
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From: Ikhlas Khan
To: [Pawar, Rahul](#)
Subject: Re: Waters Natural products program
Date: Friday, March 20, 2015 12:43:24 PM

No its not a vendor show. This is organized by Waters to see the potential in the area of natural products and where technology stands.

You will good job, don't let this opportunities pass if you get one that will help you in future

Yes olemiss has done good job but now demand is growing so fast and everyone contacting us that we can't keep up not only with works but what else comes with it like politics
ik

From: <Pawar>, Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Friday, March 20, 2015 at 9:38 AM
To: Ikhlas Khan <ikh@olemiss.edu>
Subject: RE: Waters Natural products program

I don't know what they have on mind for 2 days. Hope it not another vendor show!
There was a Senate briefing for the DNA issue some days back. I provide some materials regarding the chemical methods. Sure something good will come out of this, at least a learning experience for community and regulators. I am glad our program is UM have already done good work on this topic. Sara Hand from my office is a good speaker but I am not sure she can travel with such a short notice. Best wishes for the conference. Thanks
Rahul

From: Ikhlas Khan [<mailto:ikh@olemiss.edu>]
Sent: Friday, March 20, 2015 9:51 AM
To: Pawar, Rahul
Subject: Re: Waters Natural products program

Yes, I am glad that they contacted you. I have to be there on firstday for sure but honestly speaking I have not paid any attention to it so far. Icsb is taking all the attention and its turning to be a complicated one due to NYAG and people wants to cover that part more than regular agenda. It will be good at the end. I hope
IK

From: <Pawar>, Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Friday, March 20, 2015 at 8:44 AM
To: Ikhlas Khan <ikh@olemiss.edu>
Subject: Waters Natural products program

Good morning D. Khan

Just wanted to check with you what day are you attending the Waters Natural products program?

Thanks for recommending my name.

By the way how are the preparations for the conference coming along? Sorry I could not make it this year.

Best regards

Rahul

Rahul Pawar, Ph.D.

Office of Regulatory Science

CFSAN-FDA

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 240-402-1795

Fax: 301-436-3077

From: [Pawar, Rahul](#)
To: [Ikhlas Khan](#)
Subject: RE: Waters Natural products program
Date: Wednesday, April 22, 2015 2:43:00 PM

Ok, hope to see you Monday evening then.

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Wednesday, April 22, 2015 2:42 PM
To: Pawar, Rahul
Subject: Re: Waters Natural products program

Thanks, I will let you know my where about. At this time, I have no idea when, where what but should know soon.
ik

From: <Pawar>, Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Wednesday, April 22, 2015 at 1:02 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Waters Natural products program

Hello Dr. Khan, When are you travelling next week? Let me know if you need any help- Rahul

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Friday, March 20, 2015 12:43 PM
To: Pawar, Rahul
Subject: Re: Waters Natural products program

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Best wishes for the conference. Thanks
Rahul

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Sent: Friday, March 20, 2015 9:51 AM
To: Pawar, Rahul
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IK

From: <Pawar>, Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Friday, March 20, 2015 at 8:44 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Waters Natural products program

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By the way how are the preparations for the conference coming along? Sorry I could not make it this year.
Best regards
Rahul

Rahul Pawar, Ph.D.
Office of Regulatory Science
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-3077

From: [Pawar, Rahul](#)
To: ["SHABANA I KHAN"](#)
Subject: RE: Wishes
Date: Monday, January 5, 2015 3:26:00 PM

We all took turns falling sick during the holidays ☺ Will talk sometime, thanks

From: SHABANA I KHAN [<mailto:skhan@olemiss.edu>]
Sent: Monday, January 05, 2015 3:23 PM
To: Pawar, Rahul
Subject: Re: Wishes

Yes, we had good holidays – full of guests. Hope you also had good time. I will call you guys sometime.
Take care

Shabana Khan, Ph.D.
Principal Scientist
Room 2035 NCNPR, School of Pharmacy
University of Mississippi MS 38677
Phone: 662-9151041

From: <Pawar>, Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Monday, January 5, 2015 2:20 PM
To: Shabana Khan <skhan@olemiss.edu>
Subject: RE: Wishes

Thank you, hope you had a good holiday.

From: SHABANA I KHAN [<mailto:skhan@olemiss.edu>]
Sent: Monday, January 05, 2015 3:19 PM
To: Pawar, Rahul
Subject: Re: Wishes

Thanks Rahul and we also wish you and your family a very happy and healthy new year !

Shabana Khan, Ph.D.
Principal Scientist
Room 2035 NCNPR, School of Pharmacy
University of Mississippi MS 38677
Phone: 662-9151041

From: <Pawar>, Rahul <Rahul.Pawar@fda.hhs.gov>
Date: Monday, January 5, 2015 1:14 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>, Shabana Khan <skhan@olemiss.edu>
Subject: Wishes

Wish you and your family a Happy New Year!
Best wishes

From: [Pawar, Rahul](#)
To: ["Ikhlas Khan"](#)
Subject: RE: Wishes
Date: Friday, December 25, 2015 9:23:00 PM

Thank you Sir, we are travelling to India on Monday night!

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Friday, December 25, 2015 1:03 PM
To: Pawar, Rahul
Subject: Re: Wishes

Thanks wish you the best for 2016

Sent from my iPhone

On Dec 25, 2015, at 11:40 AM, Pawar, Rahul <Rahul.Pawar@fda.hhs.gov> wrote:

Hello,

Wish you all Happy Holidays and a Prosperous New Year!

Best regards,

(b) (6) and Rahul

From: [Pawar, Rahul](#)
To: ["BHARATHI AVULA"](#)
Subject: RE: Yatin's Email Id
Date: Monday, June 27, 2011 9:01:00 AM

No did not have any clue about swami, He might have not found time to inform me.
We are travelling on 29th and will take trainings on 30th.

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Monday, June 27, 2011 8:59 AM
To: Pawar, Rahul
Subject: RE: Yatin's Email Id

Swamy was telling me that he will call you and say goodbye.....I thought you know about it...

We have booked for Saturday (July 30, 2011)...

Best wishes
Bharathi

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Monday, June 27, 2011 7:57 AM
To: BHARATHI AVULA
Subject: RE: Yatin's Email Id

That is the only e-mail of yatin I have.
I did not know about swami till now, I missed to say bye to him. I will write to him some time, where did he join?
How is uncle and aunty say our hello to them, I hope to see them next month in san Diego, I reserved a room and flight on Friday.
Rest fine
Take care
Rahul

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Saturday, June 25, 2011 12:46 PM
To: Pawar, Rahul
Subject: RE: Yatin's Email Id

I did not get any reply from Yatin...

You should be aware that Swamy is leaving to (b) (6) for his new job on sunday 26th June 2011.

I hope everything is fine with you all..

best wishes
Bharathi

From: BHARATHI AVULA
Sent: Monday, May 23, 2011 8:24 AM
To: Pawar, Rahul
Subject: RE: Yatin's Email Id

Thank you Rahul...

Bharathi

From: Pawar, Rahul [mailto:Rahul.Pawar@fda.hhs.gov]
Sent: Monday, May 23, 2011 8:14 AM
To: BHARATHI AVULA
Subject: RE: Yatin's Email Id

(b) (6) @gmail.com
I am not sure about his phone number

From: BHARATHI AVULA [mailto:bavula@olemiss.edu]
Sent: Friday, May 20, 2011 5:50 PM
To: Pawar, Rahul
Subject: Yatin's Email Id

Dear Rahul,

Could you please email me the phone # and email id of Yatin...

Thank you
Bharathi

From: [Pawar, Rahul](#)
To: ["Xiang Fu"](#)
Subject: RE: Your pictures at Oxford ICSB.
Date: Tuesday, April 26, 2011 12:35:00 PM

Hi Fu Xiang,

I am happy you could spend some time relaxing with your wife after the hectic conference. We had a great time oxford, I think we still belong to oxford and always enjoy getting back there and meeting nice people like you.

Thank you for the pictures, it is really nice of you. A special thanks from (b) (6) too.

Keep in touch, Best regards

Rahul

From: Xiang Fu [mailto:xfu2@olemiss.edu]
Sent: Tuesday, April 26, 2011 12:19 PM
To: Pawar, Rahul
Subject: Your pictures at Oxford ICSB.

Dear Rahul,

Please see the attached pictures at the conference. Sorry for the late sending since I was in Boston to visit my wife Last week.

Have a nice day!

Best regards,

Xiang Fu
Graduate Student
Department of Pharmacognosy
School of Pharmacy
The University of Mississippi
University, MS 38677-1848
Phone: 662-915-1033 Fax: 662-915-7989

From: Jennifer S. Taylor
To: [a shakil](#); [Atish Paul](#); [Atul Jadhav](#); [chidananda swamy Rumalla](#); [Dr. Vijai K Agnihotri](#); [earla ravinder](#); [Ehab](#); [Erdal Bedir](#); [Feng Wei](#); [Hyung-in Moon \(himun68@dau.ac.kr\)](#); [Jamal Mustafa](#); [jamal mustafa mustafa](#); [Julius Ngunde Ngwendson](#); [li jing](#); [Markus Ganzera](#); [MATSUZAKI Keiichi \(matsuzaki.keiichi@nihon-u.ac.jp\)](#); [Nurdan S Duzgoren-Aydin \(naydin@njcu.edu\)](#); [Pawar, Rahul](#); [Sara Crockett](#); [Sridhar Rao Ayinampudi](#); [Toshiaki Makino \(makino@phar.nagoya-cu.ac.jp\)](#); [Vaishali Joshi](#); [vamsi.m](#); [wangwei](#); [yatin shukla](#); [Young-Whan Choi \(ywchoi@pusan.ac.kr\)](#)
Subject: Recent News
Date: Thursday, August 23, 2012 12:52:13 PM

Dr. Khan wanted me to contact you all to let you know of the passing of Rangavalli Manyan. She passed this week at her home. The arrangements are still unknown at this time. I will try to keep you posted on the details.

Thank you,

Jennifer Taylor

Jennifer Taylor
Senior Secretary
University of Mississippi
National Center for Natural Products Research
301Q Thad Cochran
P.O. Box 1848
University, MS 38677

✉ jnnfrtyl@olemiss.edu
☎ 662-915-1090
📠 662-915-7989

From: Xiang Fu
To: [Pawar, Rahul](#)
Subject: Regards from Xiang and inquiry about the job opportunity around you
Date: Monday, April 23, 2012 10:31:28 AM
Attachments: [fx CV for Olemiss.pdf](#)

Dear Rahul,

May I ask you a question about job opportunity in FDA? I am graduating this summer, but I didn't get any offer now. I talked to several people on the ICSB conference this year but it seems funding is a problem for them. So could you please give me some suggestions if you happen to know some openings around you? I attached my CV for your information. Thank you very much!

Sincerely,

Xiang Fu
Graduate Student
Department of Pharmacognosy
School of Pharmacy
The University of Mississippi
University, MS 38677-1848
Phone: 662-915-1033 Fax: 662-915-7989

From: [Pawar, Rahul](#)
To: ikhan@olemiss.edu
Subject: Request for chemical
Date: Monday, July 18, 2016 3:42:00 PM

Hello Dr. Khan,

Do you have b-O-methysynephrine in you laboratory and can you send us 5-10 mg? I remember Sampath kumar worked on it.

Thanks,

Rahul

Rahul Pawar, Ph.D.

Office of Regulatory Science/DBC/BMB

FDA/Center for Food Safety and Applied Nutrition

5100 Paint Branch Parkway

College Park, MD 20740

Tel: 240-402-1795

From: TROY J SMILLIE
To: [Pawar, Rahul](#); [VIJAYASANKAR RAMAN](#)
Subject: shipment
Date: Wednesday, September 7, 2011 11:00:19 AM

Dear Rahul,

The tracing number for your shipment is 7974 8650 4410 via fed ex. You should receive it tomorrow.

Troy Smillie, Ph.D.
Senior Research Scientist
Thad Cochran National Center for Natural Products Research
School of Pharmacy
University of Mississippi
University, MS 38677
Phone 662-915-1168
Fax 662-915-7062
<http://www.olemiss.edu/depts/pharmacy/php/sopquery3.php?id=69>

From: icsb@olemiss.edu
To: icsb@olemiss.edu
Subject: Slides from your ICSB oral presentation
Date: Thursday, April 28, 2016 5:08:56 PM

Greetings from the Oxford ICSB web team.

We would like to ask for your permission to publish your slides on the previous conferences page of our web site at <http://oxfordicsb.org>. The slides will be downloadable in the form of a locked (non-editable) PDF, converted from the Powerpoint file from your presentation.

If you would like to grant us permission, simply reply to this email. If you would like us to delete your slides from our records, please let us know that as well. If you would like to grant permission, but need to edit or remove some slides from the file, feel free to send us an edited PDF or Powerpoint file, or simply let our team know which slides to alter and we will send another email to confirm after edits are made.

Thank you so much for your contribution to the ICSB, and we look forward to hearing from you on this matter!

Regards,

Oxford ICSB Web Team

icsb@olemiss.edu

<http://oxfordicsb.org>

<http://facebook.com/OxfordICSB>

From: Jennifer S. Taylor
To: lbraun@blackmoresinstitute.org.au; pharmacog@gmail.com; gerhard.franz@chemie.uni-regensburg.de; schmeda@utalca.cl; profibi@gmail.com; moylerdavid@yahoo.co.uk; bastiaan.venhuis@rivm.nl; kuma0401@gmail.com; wendy.applequist@mobot.org; BalasubM@einstein.edu; dbudnitz@cdc.gov; Cohen, Pieter; Aeichner@usada.org; stefan@herbalgram.org; ageller@cdc.gov; Victor Navarro; joshua.sharfstein@jhu.edu; szafraj@uab.edu; gda5958@163.com; hevi@tasly.com; jishen2013@163.com; skoh@ewha.ac.kr; wangwei402@hotmail.com; ckangerhofer@yahoo.com; Rdas@Botanicals.com; loren@unpa.com; Kate Yu; catherine.rimmer@nist.gov; travis@nsf.org; Liu, Yitong; Mahmoud A. Elsohly; Handy, Sara; giorgis_isaac@waters.com; Lee, Sau; apm@gwpharm.com; Musser, Steven M; Throckmorton, Douglas C; AMAR GOPAL CHITTIBOYINA; patricia.deuster@usuhs.edu; EDWARD.Kennelly@lehman.cuny.edu; Pawar, Rahul; Sadrieh, Nakissa; Tartera, Carmen *; NATASCHA TECHEN; Welch, Cara; RRountree@thorne.com; mary@maryhardy.com; farhanja_2000@yahoo.com; txb@plantaphile.com; Paula Brown@bcit.ca; caixiong@hnctcm.edu.cn; Muhammad Iqbal Choudhary (iqbal.choudhary@iccs.edu); GurleyBillyJ@uams.edu; james.harnly@ars.usda.gov; harringp@ohio.edu; drkamil55@hotmail.com; newmean@hnctcm.edu.cn; simmier@uic.edu; cquave@emory.edu; mtims@muih.edu; james_traub@waters.com; hans.wohlmuth@integria.com; mahady@uic.edu; roe.al@pq.com; Jimmy Yuk@waters.com; a.raab@abdn.ac.uk; i_zweigenbaum@agilent.com; phil_wylie@agilent.com; SHABANA I KHAN; rphans2@uic.edu; hao@usp.org; chanduruma@yahoo.co.in; Cohen, Pieter; [Betz, Joseph M \(NIH\)](mailto:Betz, Joseph M (NIH)); [Rick Kingston \(rkingston@safetycall.com\)](mailto:Rick Kingston (rkingston@safetycall.com)); isakg-ayush@nic.in; dq-ccras@nic.in; ghazala.javed@gov.in; ravindrasinghm@gmail.com; rudi; khcbaser@gmail.com; Larry Walker; little.i.6@pq.com; zhousp@tasly.com; mark@herbalgram.org; Mahmoud A. Elsohly
Subject: Speaker information for ICSB
Date: Wednesday, April 6, 2016 5:43:48 PM
Attachments: [Agenda 3-30-16 Book format.docx](#)

Dear Speaker,

We are so excited that we are so close to the beginning of our information filled event. Again, we thank you for your important contribution. If you have not already, please send me your travel information. If you have already sent it, thank you again.

We would like to invite you to join us for supper on Sunday evening (April 10th), if your itinerary permits, at **Toyo Japanese Sushi Bar & Hibachi** (2305 Jackson Ave W #207, Oxford, MS 38655) from **5-9pm**. If you need someone to pick you up at your hotel, please call me at the number listed below. We will have a shuttle going/coming throughout the evening. If your shuttle drop off in Oxford is during this time, please ask the driver to drop you off for the meal and we will make sure you get back to your hotel.

If you have a very late flight and would like me to have a meal waiting for you at the hotel, please let me know.

Please bring your talk with you on a flash drive or you can submit your presentation files here (any time leading up to the conference):

<https://www.dropbox.com/request/FB2ipB7E42dRLTLFpXoq> . The files will be private, and you do not need a dropbox account to upload. Please name your files so that they contain your full name (e.g. Ikhlas_Khan_presentation.pptx). This way we can have your slides loaded and ready to go for your sessions. If you have issues with or questions about the uploading process, please email us.

I am attaching a copy of the final agenda. Please be sure to note your session time and talk duration. The talk time vary per session.

Here is my cell phone number in the event you need any assistance + (b) (6) . Please do not hesitate to call.

I wish you safe travels,

Jennifer Taylor

Jennifer Taylor

Program Coordinator

University of Mississippi

National Center for Natural Products Research

3012 Thad Cochran

P.O. Box 1848

University, MS 38677

✉ jnnfrtyl@olemiss.edu

☎ 662-915-1090

📠 662-915-7989

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From: icsb@olemiss.edu
To: icsb@olemiss.edu
Subject: Special thanks from the ICSB
Date: Friday, April 15, 2016 3:32:22 PM

Greetings,

Dr. Ikhlas Khan and the entire ICSB team would like to extend our deepest gratitude to our speakers. Your efforts and your intellects helped us to deliver another successful and highly interesting scientific program for our attendees! Without each and every one of you, we very simply could not bring together such a wonderful gathering of industry leaders, academics and government representatives to inform, entertain, and bring them together to further the national and international conversation on natural products research.

Don't forget, next year's conference will be held from April 3rd – 6th, 2017, and we look forward to sharing it with you all!

Thank you once again,

Dr. Ikhlas Khan & the ICSB Team

icsb@olemiss.edu

<http://oxfordiscb.org>

<http://facebook.com/OxfordICSB>

From: [Pawar, Rahul](#)
To: ["ICSB@olemiss.edu"](mailto:ICSB@olemiss.edu)
Subject: Submission of Poster
Date: Thursday, December 2, 2010 3:54:00 PM
Attachments: [Acacia-ICSB2011-final.doc](#)

Dear Organizers,
Please find my abstract attached for presentation as a poster at the ICSB meeting.
Hope to see you in April
Happy Holidays
Rahul

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 301-436-1795
Fax: 301-436-2622

From: 3884543.4315017
To: [Pawar, Rahul](#)
Subject: Thanks for registering!
Date: Tuesday, January 3, 2017 8:26:59 AM

Thanks for your interest in the 17th Annual Oxford ICSB, Apr 03 - 06, 2017
Your login information is:

email: rahul.pawar@fda.hhs.gov

Password: (b) (6)

Please keep them for your records. Don't forget to mark your submissions as final before the submission deadline of December 1st, 2015 to be considered for presentation at the Oxford ICSB. If you have any questions, contact us at icsb@olemiss.edu.

From: ICSB Web Team
To: [Pawar, Rahul](#)
Subject: Thanks from the Oxford ICSB
Date: Friday, April 15, 2016 3:36:27 PM

ICSB Header Generic



Joint Meeting with 16th Annual International Conference on the Science of Botanicals (ICSB) & 5th Interim American Society of Pharmacognosy (ASP)

Greetings,

To all ICSB/ASP participants, presenters, volunteers and friends, the ICSB Team would like to extend our heartfelt thanks for your assistance in making this year's conference another rousing success!

We had a wonderful meeting, with representation from 5 continents and more than twenty countries around the world, more than 50 oral presentations and nearly 200 posters. We also saw a high level of lively discourse in our panel discussions and question and answer sessions. None of this would be possible without all of your support through the years, and into the future!

We hope that you all enjoyed this year's scientific programming as well as our schedule of social events and outings. We look forward to seeing you all again for our future meetings. Don't forget, the 17th Annual Oxford ICSB is scheduled for April 3rd - 6th, 2017! If you have any comments, questions, or suggestions from this year or for our upcoming meetings, don't hesitate to contact us at icsb@olemiss.edu.

Thanks again,

The ICSB Team

icsb@olemiss.edu

<http://oxfordicsb.org>

<http://facebook.com/OxfordICSB>

From: icsb@olemiss.edu
To: [Pawar, Rahul](#)
Subject: Upcoming ICSB-ASP
Date: Monday, March 21, 2016 1:03:14 PM
Attachments: [Agenda 3-21-16 Book format.pdf](#)

I just wanted to touch base with you about our upcoming ICSB-ASP meeting. Please find our tentative agenda attached. Would you please review and see if all of your information is correct before we send it to the printer. It would be great if you would send me copy of your itinerary and you hotel information? Will you need the shuttle service? I am listing my contact information below in case you need anything or have any questions. I will be available most anytime.

Jennifer Taylor
662-915-1090 OFFICE
(b) (6) CELL

International Conference on the Science of Botanicals
National Center for Natural Products Research
University MS 38677
Phone 662-915-1090
Fax 662-915-7989
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www.OxfordICSB.org

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From: [Pawar, Rahul](#)
To: ["bavula@olemiss.edu"](mailto:bavula@olemiss.edu)
Subject: update
Date: Friday, July 30, 2010 10:32:05 AM

This is from Murthybulletin

Looks like good new can come to you soon.

1. Priority Date Movement Helps I-485 Approvals and New Filings

As regular **MurthyDotCom** and **MurthyBulletin** readers are aware, the U.S. Department of State (DOS) visa bulletin cutoff dates for the employment-based, second preference category, often referred to as EB2, advanced significantly for Indian nationals in July 2010. The immigrant ("green card") department of the Murthy Law Firm reports that this movement is having a clear impact on the approval of many adjustment-of-status (I-485) cases.

Approvals of I-485s with Current Priority Dates

Mail delivered to the Murthy Law Firm brings good news for many of our clients with I-485 cases pending in the EB2 category. We have been receiving I-485 approvals for these individuals throughout the month of July 2010. This is expected to continue in August 2010, as the cutoff date for EB2 India shown in the August 2010 DOS Visa Bulletin, moved forward yet again.

Background on Many Pending I-485s

The I-485 approvals are primarily for cases filed in July, August, and September 2007. Information on the July 2010 and August 2010 Visa Bulletins is available in our articles, [July 2010 Visa Bulletin: Good News for EB2 India](#) (18.Jun.2010), [August 2010 Visa Bulletin: More Good News for EB2s](#) (16.Jul.2010). This trend could continue through September 2010, if that visa bulletin contains continued visa number availability for EB2 for nationals of India. The reason the priority dates have remained current is the DOS's goal to use the entire quota of immigrant numbers available in each fiscal year. The U.S. Citizenship and Immigration Services (USCIS) fiscal year ends September 30th annually.

Murthy Follows Up with USCIS on Pending I-485 Cases

There are many cases that are potentially eligible for approval in July 2010. This group will expand in August 2010. While the impact of the July Visa Bulletin on our cases was almost immediate, many individuals still wait and hope that their cases finally will be approved while the priority dates remain current. At the Murthy Law Firm, we know how important it is for our clients to obtain their I-485 approvals. We routinely follow up with the USCIS on all cases that have become current under the July and August Visa Bulletins.

USCIS WebSite Reveals Information on Pending I-485s

The volume of pending I-485 cases is reported in charts available on the [USCIS WebSite](#). We have previously discussed these charts in articles, including [USCIS Update on Pending I-485s](#). The most recent [charts](#) available are dated May 27, 2010. The next update of the charts should show some significant changes in the number of EB2 cases for nationals of India and China with pending I-485 cases with priority dates in 2005 and early 2006.

From: BHARATHI AVULA
To: [Pawar, Rahul](#)
Subject: vacation
Date: Tuesday, November 26, 2013 1:24:44 PM

Tomorrow I am traveling to India and will be back in January 2014.

Happy Thanksgiving and Happy New Year to you all....

bharathi

From: [Pawar, Rahul](#)
To: ikhana@olemiss.edu
Subject: Wishes
Date: Monday, July 28, 2014 2:15:00 PM

Happy it to you and your family!!

Rahul Pawar Ph.D.
Division of Bioanalytical Chemistry
CFSAN-FDA
5100 Paint Branch Parkway
College Park, MD 20740
Tel: 240-402-1795
Fax: 301-436-2622

From: [Pawar, Rahul](#)
To: ["mstrawn@olemiss.edu"](mailto:mstrawn@olemiss.edu)
Subject: Wishes
Date: Friday, May 28, 2010 12:25:08 PM

Dear Kathy,

I don't know if this is your last day of work but we could like to wish you a happy and healthy retired life. You will be dearly missed at the NCNPR and Dr Khan's group and I don't think any person will be able to fill your shoes.

Keep in touch and all the best

Love

Rahul, (b) (6)

From: 3884543.4315017
To: [Pawar, Rahul](#)
Subject: Your submission is finalized!
Date: Tuesday, December 15, 2015 4:21:16 PM

Rahul Pawar,

Thanks for your submission: Assessment of the Authenticity of Herbal Dietary Supplements: Comparison of chemical and DNA barcoding methods.

Your abstract has been marked as final and will be considered for presentation at the Oxford ICSB. You will be notified if your abstract is accepted after the submission deadline. If you have any questions, contact us at icsb@olemiss.edu.

From: 3884543.4315017
To: [Pawar, Rahul](#)
Subject: Your submission is finalized!
Date: Tuesday, January 3, 2017 1:11:16 PM

Rahul Pawar,

Thanks for your submission: Validation of an LC-MS/MS Method for Analysis of Anti-diabetic, Anti-obesity, and Cholesterol-lowering Drugs in Botanical Dietary Supplements Labelled for Blood Sugar Management
Your abstract has been marked as final and will be considered for presentation at the Oxford ICSB. You will be notified if your abstract is accepted after the submission deadline. If you have any questions, contact us at icsb@olemiss.edu.

Tsai, Victoria

From: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Sent: Thursday, August 03, 2017 4:28 PM
To: Moghaddam, Sarvin
Cc: Vukmanovic, Stanislav; Sadrieh, Nakissa; Ikhlas Khan
Subject: RE: products with Aloe ingredient

Thank you and will be very helpful.

From: Moghaddam, Sarvin [mailto:Sarvin.Moghaddam@fda.hhs.gov]
Sent: Thursday, August 3, 2017 3:24 PM
To: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Cc: Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>; Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Subject: products with Aloe ingredient

Hi Amar,
Attached please find the products with Aloe ingredients. Please let me know if you need any additional information.
Both files represent the same products in different formats.

Thank you
Sarvin

Tsai, Victoria

From: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Sent: Wednesday, February 28, 2018 4:05 PM
To: Moghaddam, Sarvin
Subject: RE: question

See my initial thoughts below. If needed, we can discuss over phone. Thanks, -Amar

From: Moghaddam, Sarvin [mailto:Sarvin.Moghaddam@fda.hhs.gov]
Sent: Wednesday, February 28, 2018 12:12 PM
To: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: question

Hi Amar,
Hope all is well.

I have been asked to provide some inputs to the following questions and the truth is I don't have any hands-on experience on any kind of in-vitro testing. I have been trained as a computational chemist.

The draft I am reviewing is about test guideline for "chemical mixtures testing" and applicability domain of a test method may be in contradiction with its potential use for testing mixtures and the concern is the technical applicability, and its utility to get meaningful results, following the testing of a chemical mixture

It's challenging filed (testing of chemical mixtures) and very much needed. If successful, the obtained data is much more relevant to realistic scenarios (fragrances, essential oils, multi-ingredient cosmetic formulations, etc.).

And the questions I have been asked are:

{Is it desirable and possible to set-up a central repository for chemical mixtures?

In my opinion its tedious process and outcome (testing data) is highly variable and time-dependent. The main reason is that each chemical half-life is different and stability of each chemical is influenced by other chemicals present in mixture. In some incidences, we noticed more stable due to synergistic interactions (terpenes with and without antioxidants). So, having central repository may not be useful?

What would be the necessary information elements for each chemical mixture that would need disclosure to help in defining the applicability domain of individual test method validated?

Limited literature data with alternative methods and their applicability to mixtures. Lot of academic/industry researchers are working towards to this goal. Based on available data, KeratinoSens and HTS-DCYA appear to be dependable. The HTS-DCYA method is not a validated method. The bottom line, we need more scientific data.

What are the means to mobilise to achieve this goal?

As I mentioned earlier, we need more alternative methods data to agree/disagree for mixtures. Like pilot study with defined mixtures (chemical finger printing, quantitation and stability established; negative and positive sensitization potential mixtures)

Who should be involved? Who should be the key players involved in such initiative if it were to be implemented?}

I would say both industry, academicians and regulators (for me being from academic institute). Industry can follow the regulatory agencies accepted methods and academic institutes can explore the innovative methods along with accepted methods. Harmonized efforts from Regulators.

I totally can understand that you might have some other deadlines at the moment, but after talking to Stan, we thought, it does not hurt to ask you and see if you can provide any input to the above questions.

Thank you
Sarvin

Department of Health and Human Services
Public Health ServicesReview Group Type Activity Grant Number
1U01FD004246**Grant Progress Report**Total Project Period
From: 09/15/2011 Through: 08/31/2011
Requested Budget Period
From: 09/1/2014 Through: 08/31/2015

1. TITLE OF PROJECT

Science Based Authentication of Dietary Supplements

2a. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR

(Name and address, street, city, state, zip code)

Khan, Ikhlas A.
120 Faser Hall/NCNPR
School of Pharmacy
University of Mississippi
University, MS 38677

2b. E-MAIL ADDRESS

ikhlan@olemiss.edu

2c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT

National Center for Natural Products Research

2d. MAJOR SUBDIVISION

School of Pharmacy

2e. Tel: 662-915-7821

Fax: 662-915-7989

3a. APPLICANT ORGANIZATION

(Name and address, street, city, state, zip code)

The University of Mississippi
Office of Research and Sponsored Programs
100 Barr Hall PO Box 907
University, MS 38677

3b. Tel: 662-915-7482

Fax: 662-915-7577

3c. DUNS: 067713560

4. ENTITY IDENTIFICATION NUMBER

1646001159A1

6. HUMAN SUBJECTS ☒ No ☐ Yes6a. Research
Exempt☒ No ☐ YesIf Exempt ("Yes" in
6a):
Exemption No.If Not Exempt ("No" in
6a):
IRB approval date

5. NAME, TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL

Mickey McLaurin, Director of Sponsored Programs
Office of Research and Sponsored Programs
100 Barr Hall or PO Box 907, University MS 38677

6b. Federal Wide Assurance No.

Tel: 662-915-7482

Fax: 662-915-7577

6c. NIH-Defined Phase III

Clinical Trial ☒ No ☐ Yes

E-MAIL: research@olemiss.edu

7. VERTEBRATE ANIMALS ☐ No ☒ Yes

7a. If "Yes," IACUC approval Date 06-20-12

7b. Animal Welfare Assurance No. A3356-01

10. PROJECT/PERFORMANCE SITE(S)

Organizational Name: University of Mississippi

DUNS: 067713560

8. COSTS REQUESTED FOR NEXT BUDGET PERIOD

8a. DIRECT \$1,798,535

8b. TOTAL \$2,500,000

Street 1: 120 Faser Hall/NCNPR

Street 2: School of Pharmacy

9. INVENTIONS AND PATENTS ☒ No ☐ YesIf "Yes," ☐ Previously Reported
☐ Not Previously Reported

City: University

County: Lafayette

State: MS

Province:

Country: USA

Zip/Postal Code: 38677

Congressional Districts: MS-001

11. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Item 13)

Mickey McLaurin, Director of Sponsored Programs Administration

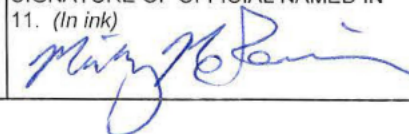
TEL: 662-915-7482

FAX: 662-915-7577

E-MAIL: research@olemiss.edu

12. Corrections to Page 1 Face Page

13. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN
11. (In ink)

DATE

5/30/14

			Salary Req.	Fringe	Total
Personnel					
PI	Ikhlas Khan	45%	(b)	(6)	
Co-PI	Larry Walker				
Principle Res.					
Scientist	Shabana Khan	23%			
Post Doc	Amira Wanas	50%			
Sr. Research					
Scientist	Bharthi Avula	70%			
Sr. Research					
Scientist	Yan Hong Wang	100%			
Res. Scientist	Natascha Techen	100%			
Res. Scientist	Zulfiqar Ali	25%			
Sr. Research					
Scientist	Amar Chittiboyina	50%			
Res. Scientist	Guoyi Ma	100%			
Res. Scientist	Ahmad Osman	100%			
Assoc. Res. Scientist	Jianping Zhou	53%			
Post Doc	Vijayasankar Raman	100%			
Post Doc	Mei Wang	70%			
Post Doc	Cristina Avonto	100%			
Post Doc	Vamshikrishna Manda	100%			
Post Doc	Zhihao Zhang	100%			
Post Doc	Min Hye Yang	100%			
Assoc. R&D Biologist	Helaina Craig	100%			
	Satyanarayanaraju				
Post Doc	Sagi	100%			
Post Doc	Pradeep Lasonkar	100%			
Post Doc	Iffat Parveen	100%			
R&D Botanist	Lal Jayaratna	100%			
R&D Data Analyst	Steven Hopper	100%			
Project Coordinant	Gray Dale	25%			
Program Coordinator	Jennifer Taylor	100%			
Hourly Wages		100%			
Graduate Students					
(4)		100%			
Total Salaries and FB					\$1,241,419
equipment					\$150,000
supplies					\$165,181
travel					\$40,000
contractual services					\$140,000
MOBOT					\$54,296
Subtotal					\$1,790,896
F&A 44%					\$709,104
Total Request					\$2,500,000

BUDGET JUSTIFICATION	GRANT NUMBER 1U01FD004246
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Provide a detailed budget justification for those line items and amounts that represent a significant change from that previously recommended. Use continuation pages if necessary.

A. PERSONNEL: \$1,241,419

Faculty and Professional Staff

PI, Dr. Ikhlas A. Khan, Assistant Director and Research Professor, National Center for Natural Products Research, Principal Investigator will devote 45% of his time to this program. Dr. Khan will be the contact point with NCNPR for interactions with FDA scientists. Dr. Khan has extensive research project administrative experience, has served as PI of several projects. Dr. Khan is currently working as a program director of FDA program at the Center. He works directly with Dr. Walker on a daily basis for scientific direction of major portions of NCNPR research efforts.

Co-Investigator, Dr. Larry A. Walker, Director, National Center for Natural Products Research, Co-Principal Investigator will provide the time & effort necessary for the overall administrative direction of the program. Dr. Walker will organize and coordinate the Steering Committee operation, and work closely with FDA officials and University administration to ensure administrative compliance and performance. No costs will be incurred to the grant for Dr. Walker's support.

CURRENT BUDGET PERIOD	FROM 09/01/13	THROUGH 08/31/14
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Explain any estimated unobligated balance (including prior year carryover) that is greater than 25% of the current year's total budget.
N/A

BUDGET JUSTIFICATION CONTINUATION

Faculty and Professional Staff Continued

Principle Research Scientist (Dr. Shabana I. Khan) - 23% effort. Dr. Khan will be responsible for direction of the efforts at UM to evaluate toxicological parameters for the natural products and botanical extracts. She will commit to the project. Dr. Khan's training is in biochemistry, and she has worked for many years in the screening development with natural products. She will supervise the efforts of the toxicology research associates.

Sr. Research Scientist, Synthetic Chemist (Dr. Amar Chittiboyina) – 50% effort. Dr. Chittiboyina will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards. Dr. Chittiboyina will be responsible for all scientific aspects of data management for the project. Dr. Chittiboyina will coordinate particularly with the botanists, geneticist, analytical and isolation chemistry investigators, as well as with FDA scientists involved in the project, to develop and modify the data management workflow.

Sr. Research Scientist, Analytical Chemist. (Dr. Bharathi Avula) – 70% effort. Research Scientist, National Center for Natural Products Research will be responsible for analytical method development. Dr. Avula is a trained pharmacist and analytical chemist. She has developed expertise over the years in analysis of botanicals. She will also be supervising Dr.'s Yan Hong Wang, and Mei Wang in developing relevant analytical methods.

Senior Research Scientist, Analytical Chemist. (Dr. Yan Hong Wang) – 100% effort. Dr. Wang will be responsible for analytical method development. Dr. Wang is a natural products chemist with analytical background. He has developed an extensive expertise over the years in the analysis of botanicals.

Research Scientist, Plant Genetics (Dr. Natscha Tehen) – 100% effort. Dr. Tehen will be responsible for plant genetics approaches applied to the authentication of botanical dietary supplements. She is experienced in all the molecular techniques needed to accomplish this task.

Research Scientist, Isolation Chemist (Dr. Zulfiqar Ali) – 25% effort. Dr. Ali will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Research Scientist, Biologist (Dr. Gouyi Ma) – 100% effort. Dr. Ma will be responsible for the development of in-vitro assays to assess the toxicological profile of botanicals

Research Scientist, Chemist – (Ahmad Osman) 100% effort. This individual will be responsible for isolating and identifying compounds of interest from various botanical sources. Additionally, this person will be instrumental in compiling scientific information with regard to identification techniques (analytical, NMR, etc) for the authentication of botanical dietary supplements for the purpose of populating a dynamic web portal to assist FDA researchers and other stakeholders.

Associate Research Scientist, Isolation Chemist (Dr. Jiaping Zhao) – 53% effort. Dr. Zhao's responsibility will be to prepare extracts of selected botanicals, develop NMR identification/authentication techniques, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Postdoctoral Research Associate, Botanist (Dr. Vijayasankar Raman) – 100% effort. Dr. Raman will be working on macro and microscopy of botanicals, needed for species authentication. Microscopy provides an understanding of anatomical features of a plant, which is instrumental in differentiating the species and also in determining potential adulterants.

Post Doctoral Research Associate, Analytical Chemist (Dr. Mei Wang) – 70% effort. Dr. Wang will develop analytical methods for the purpose of identifying botanical dietary supplement samples. Dr. Wang has several years of experience in developing analytical GC techniques for the purpose of analysis and quantitation.

Post Doctoral Research Associate, Chemist (Dr. Cristina Avonto) – 100% effort. Dr. Avonto will be responsible for isolating marker compounds and bioactive constituents from botanicals. Additionally Dr. Avonto will perform analytical profiling of botanicals using various GC techniques.

Post Doctoral Research Associate, Biologist (Dr. Vamshikrishna Manda) – 100% effort. Dr. Manda will be responsible for the development of in-vitro assays to assess the safety of dietary supplement ingredients as well as ADMET evaluation of various constituents.

Post Doctoral Research Associate, Isolation Chemist (Zhihao Zhang) – 100% effort. Dr. Zhang will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Post Doctoral Research Associate, Isolation Chemist (Dr. Min Hye Yang) – 100% effort. Dr. Yang will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Assoc. R&D Biologist (Helaina Craig) – 100% effort. Ms. Craig will help the senior scientists on animal based in vivo work for behavioral and hepatotoxic studies on the botanical of interest.

Post Doctoral Research Associate, Isolation Chemist (Dr. Satyanarayanaraju Sagi) – 100% effort. Dr. Sagi will develop analytical methods for the purpose of identifying botanical dietary supplement samples. Dr. Sagi has several years of experience in developing analytical HPTLC/LC techniques for the purpose of analysis and quantitation.

Post Doctoral Research Associate, Isolation Chemist (Dr. Pradeep Lasonkar) – 100% effort. Dr. Lasonkar will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Post Doctoral Research Associate, Plant Genetics (Iffat Parveen) – 100% effort. Dr. Parveen will be responsible for plant genetics approaches applied to the authentication of botanical dietary supplements. She will assist and train under Dr. Tehen for the molecular techniques needed to accomplish the proposed work.

Post Doctoral Research Associate, Analytical Chemist (Amira Wanas) – 50% effort. Dr. Wanas will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

R&D Botanist (Lal Jayaratna) – 100% effort. This individual is responsible for maintaining the living collection of the centers medicinal plant garden. Additionally this individual prepares voucher specimens, maintains the programs seed bank and provides assistance/tours for training sessions and workshops.

R&D Data Analyst – (Steven Hopper) 100% effort. This individual will be responsible for the development of a botanical dietary supplement information portal for the dissemination of relevant scientific data and FDA Dietary Supplement cGMP information. In addition to this he will be aiding in the data labeling, collection/reporting efforts for this project.

Project Coordinator (Gray Dale) - 25% effort. Mr. Dale is responsible to the PIs, to allow for adequate follow-up with reports, budgets and travel. Mr. Dale also provides vital logistical support for ICSB conference.

Technical and Administrative Staff

Program Coordinator (Jennifer Taylor) - 100% effort. Ms. Taylor is responsible to the PIs, to allow for adequate follow-up with reports, publications, file documentation, sample tracking, etc. Ms. Taylor also provides vital logistical support for workshops, training sessions and conferences.

NOTE: The position of Program Coordinator and Project Coordinator is normally not allowed as direct costs under OMB circular A-21. However, we are requesting these direct costs be allowed due to the large scope of the project and the number of personnel to be managed and supported. This position is easily allocable to the project, and are reasonable given the size and nature of the project.

Hourly Wages – Hourly wage support (\$17,000) will be utilized for temporary and student workers employed in support of the project. These may be utilized in support of administrative or research activities, ranging from filing and data entry to field/greenhouse or laboratory assistance.

Graduate Students (4) – The graduate students will receive training in natural products chemistry, analytical chemistry. These students will be enrolled in the Ph.D. program in the Department of Pharmacognosy, or one of the other academic Departments in the school of Pharmacy (\$40,000).

Fringe Benefits

Fringe benefits for faculty and staff are calculated at the University's standard rate of 32.75% of salary. Fringe benefits for students (graduate or undergraduate) are calculated at the University's standard rate of 8% of wages.

Increase for additional Years:

Inflationary increases of 3% per year have been included for year for personnel positions

B. CONSULTANT COSTS: None

C. EQUIPMENT: \$150,000

These funds are to support the capacity for analytical work that is required for the success of the program. These funds will be utilized to either purchase new complete systems or to upgrade other existing equipment such as HPLC, GC, NMR or MS.

D. SUPPLIES: \$ 165,181

Primary commodity expenditures for the project will be for:

HPLC columns \$22,947

NMR/MS supplies (tubes, gases, columns) \$13,700

Microscopic supplies (slides, stains, optics, mounting preparation) \$6,700

Chromatographic supplies (TLC plates, chromatographic media, solvents) \$50,834

Mol. Biology supplies \$30,000

Botanical collection/storage materials \$13,000

Garden/greenhouse tools/supplies \$12,000

Books, databases other reference materials \$12,000

Computer supplies \$4,000

Sub Total: \$165,181

E. TRAVEL: \$40,000

Included in this category are:

Travel for FDA Dietary Supplement GMP training sessions.

Establishing collection arrangements and collaborations (National and International)

Travel to/from Washington or other COE's for meetings with FDA officials

Travel costs related to hosting workshop/conference (National and International)

F. CONTRACTUAL SERVICES: \$140,000

Estimated expenditures in this category include contractual/professional services for:

Collection & documentation of plant material \$9,000

scale-up extraction/isolation \$ 9,000

taxonomic verifications \$6,000

maintenance contracts/repairs for analytical equipment \$39,500

software/upgrades for analytical equip. \$8,000

shipping, mailing costs \$4,000

Sub-Total: \$75,500

Estimated expenses for hosting conference:

Printing/PR \$3,500

Speaker reimbursements (28 @ 1,500) \$42,000

Dinners/breaks \$11,000

Staffing \$8,000

Sub Total: \$64,500

G. SUBCONTRACT: \$54,296

A subcontract with Missouri Botanical Garden will be in place in the amount of \$54,296. Missouri Botanical Garden will be collecting the specimens for this project and will be providing expertise on identification of the specimens. Dr. Rainer Bussmann, the director and curator of the William L. Brown Center for Plant Genetic Resource (WLBC), will be the Missouri Botanical Garden representative working on the project.

H. INDIRECT CHARGES: \$ 709,104

Indirect costs are calculated in accordance with The University of Mississippi's rate agreement with DHHS, dated September 12, 2011. Indirect costs for research are calculated at 44.0% of Total Direct Costs less equipment, tuition remission, and the portion of each sub-grant or subcontract in excess of \$25,000.

Program Director/Principal Investigator (Last, First, Middle): **Khan, Ikhlas A**

PROGRESS REPORT SUMMARY	GRANT NUMBER 1U01FD004246	
	PERIOD COVERED BY THIS REPORT	
	FROM 09/1/2013	THROUGH 08/31/2014
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Khan, Ikhlas A		

APPLICANT ORGANIZATION
The University of Mississippi

TITLE OF PROJECT (Repeat title shown in Item 1 on first page)
Science Based Authentication of Dietary Supplements

A. Human Subjects (Complete Item 6 on the Face Page)		
Involvement of Human Subjects	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change
B. Vertebrate Animals (Complete Item 7 on the Face Page)		
Use of Vertebrate Animals	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change
C. Select Agent Research	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change
D. Multiple PD/PI Leadership Plan	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change
E. Human Embryonic Stem Cell Line(s) Used	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

None

Progress Report Summary

A. Specific Aims

Under the provisions of the 20 years of DSHEA, the FDA has primary responsibility for ensuring that appropriate regulatory actions are taken against marketed products that present significant health risks or bear false or misleading label claims. Foundational to the evaluation of such risks and claims is an adequate scientific base for decision-making. For botanical dietary supplements (BDS), development of such a science base is especially problematic because of several unique features, including the complexity of the constituents, variability of sourcing, lack of availability of reference materials, lack of good manufacturing controls and rapidly expanding use in the marketplace. In 2010 the FDA fully implemented the current good manufacturing practices (cGMP) for the botanical dietary supplement industry placing the onus of “botanical identity and authenticity” on the manufacturers of botanical dietary supplements. However, these cGMP’s have in many ways increased the complexity as to what constitutes a “scientifically valid method” for the determination of the identity and authenticity of botanical materials. To address these complex issues surrounding botanical identity, authenticity, safety and toxicity and more importantly, in continuation of our cooperative agreement with the FDA, the NCNPR has established the following specific aims to facilitate the research needs of the FDA:

1. Assist in the identification and development of a list of BDS and botanical ingredients based on safety concerns, trends, and knowledge of botanicals being marketed in the U.S., to prioritize for further research.
2. Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to FDA, for the assessment of their adulteration, safety and toxicity.
3. Exchange technical and scientific information, analytical methods, and reference material with FDA scientists and other stakeholders.
4. Collaborate with FDA scientists in research areas of mutual interest.
5. Coordinate scientific workshops and conferences on BDS-related topics of public health relevance to address high priority science and awareness of emerging problems associated with botanicals to the public.

B. Studies and Results:

Aim 1: Assist in the identification and development of a list of BDS and botanical ingredients based on safety concerns, trends, and knowledge of botanicals being marketed in the U.S., to prioritize for further research.

Since the inception of the cooperative agreement between the NCNPR and the FDA in 2001, there have been numerous occasions for interactions between the two organizations. These have included (yearly) formal site visits by the FDA program officers, interactions during the annual International Conference on the Science of Botanicals (ICSB) and several conference calls and email exchanges. In addition to these interactions, over the past year the NCNPR has hosted five training sessions with the Office of Regulatory Affairs (ORA) to provide hands-on training to FDA inspectors for cGMP compliance issues associated with BDS’s (FD340). These training sessions have provided an opportunity for the programs project officer (Dr. Daniel Fabricant) and his colleagues to visit the NCNPR and stay abreast of the Center’s ongoing developments. As always, researchers at the NCNPR are receptive to areas of study that are deemed necessary by the FDA for this project.

Aim 2: Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to FDA, for the assessment of their adulteration, safety and toxicity.

The availability of authenticated reference materials for botanicals is an essential first step in the development of analytical methodologies for the identification and quantitative analysis of botanical product(s). The NCNPR has been very successful in placing formal agreements with several international academic and governmental

institutions including Pakistan, India (FRLHT), South Africa, Central/South America, China with the development of the Sino-US TCM Research Center and the established Center for Research in Indian Systems of Medicine (CRISM - www.CRISM.net) with the departments of AYUSH and CSIR in India. The NCNPR has also established a Memorandum of Understanding (MOU) with the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Brazil and the Natural Products Utilization Research Unit (NPURU), USDA located in Oxford Mississippi. Recently the NCNPR has cultivated a productive relationship with the Chinese Pharmacopeia and Chinese FDA in order to obtain relevant authenticated Traditional Chinese Medicines (TCM's) and pertinent purified constituents for authentication purposes. With this mechanism, NCNPR has acquired more than 150 new constituents over the past year and the collaboration would facilitate NCNPR to aid in populating a botanical information portal for CFSAN/FDA and expanding the in-house repository. In 2013, as a part of collaboration, more than 125 samples of authenticated tea tree oils were obtained from Southern Cross University, Australia to assess the safety, development of authentication techniques and to provide samples for possible allergen testing for CFSAN's cosmetic program. Lastly, NCNPR established an agreement with Tshwane University of Technology, Pretoria, South Africa to exchange the traditional practices based on botanicals endemic to South Africa such as *Sutherlandia frutescens*, *Hoodia gordonii* and other related plant materials of interest to the FDA.

From these and other collaborative relationships, the NCNPR has been able to acquire over 9500 plant samples and herbal extracts, representing approximately 5000 species over the duration of this project. There is a continuous effort in acquiring commercial samples as well for authentication purposes in addition to assuring that the developed identity testing techniques for this project are applicable for finished products in various formularies. Taking all these sample types into account, there are over 16,000 samples within the NCNPR-FDA repository. All the plants and samples collected for this program have been assigned an inventory number and are cataloged in the NCNPR repository and voucher specimens of authentic samples are also maintained at the Thomas M. Pullen Herbarium in the Department of Biology at the University of Mississippi.

In addition to the repository, the NCNPR has a newly renovated Medicinal Plant Garden that maintains more than 300 species for selected growing (field, greenhouse and shade houses). The new facilities consist of two main buildings (4,362 sf. and 4,290 sf.) and four additional support buildings and structures sitting on approximately 5.25 acres of land. The new facility was dedicated as the Maynard W. Quimby Medicinal Plant Garden on April the 15th 2013 as a part of the 12th ICSB. The Medicinal Plant Garden has developed significant collaborations with seed banks from 40 institutes around the world in order to exchange medicinally important germplasms. This seed bank effort has yielded a collection of over 1530 species to date. In addition, the garden personnel are preparing herbarium voucher specimens from the living collection and have started collecting samples for a DNA tissue bank. Over the course of this program, the garden provided 320 authentic reference samples from the living collection for the Center's research efforts. The Medicinal Plant Garden is also registered under the Botanic Gardens Conservation International and is a member of the American Association of Botanical Gardens and Arboreta and International Plant Exchange Network (IPEN). Overall, this new facility provides not only an invaluable resource for propagating and sourcing botanicals of interest but also provides a training facility for FDA/ORA courses on identification of botanical of interest.

Isolation of reference compounds: The isolation of standard compounds is required to develop analytical methods, quantify individual compounds in extracts and to establish analytical fingerprints in support of authenticity, quality, safety, and toxicity studies. Scientists at the NCNPR have isolated a number of compounds from species such as *Mitragyna speciosa*,¹ *Dioscorea villosa*,² *Dioscorea cayenensis*,³ *Dioscorea nipponica*,⁴ *Matricaria recutita* L., *Anthemis nobilis*, and *Lepidium meyenii*⁵ (Maca). It is through these continued efforts that the NCNPR scientist have isolated several novel compounds as well as known analogs for analytical finger printing development for authentication purposes as well as safety analysis.

Synthesis and procurement of compounds of interest: Under certain situations, synthesis of reference compounds is also undertaken at NCNPR wherein isolation of marker compounds was laborious and time consuming. Several sympathomimetic amines such as *N,N*-diethylphenethylamine, *N,N*-dimethylphenethylamine, phenethylamine, cocularine were synthesized from commercially available raw

materials on a bulk scale. In addition to large scale synthesis, several single enantiomers were synthesized for the development of analytical methods to understand the origin (synthetic/natural) of compounds of interest.

Analytical method development and metabolomic profiling: In the past year we have focused on developing several analytical methods for the determination of authenticity BDS's of interest. These studies include the development of UPLC (UV, ELS and MS), Supercritical Fluid Chromatography (SFC), standard HPLC/HPTLC analytical methods as well as using proton NMR for metabolomic profiling for common botanicals including *Matricaria recutita* L., (German chamomile) *Anthemis nobilis*, (Roman chamomile), *Chrysanthemum morifolium* (Chinese chamomile); determination of coumarin in Cinnamon species⁶ (*Cinnamomum verum*, *C. cassia*, *C. loureiroi*, and *C. burmannii*); *Terminalia* species⁷⁻⁹; *Dioscorea* species (*Dioscorea villosa* L., *D. cayennensis* Lam., *D. rotundata*, *D. opposita*, *D. caucasica*, *D. bulbifera*, *D. deltoidea*, *D. quaternata*); *Mitragyna speciosa*¹⁰,¹¹ Korth; *Dendrobium nobile*; pyrrolizidine alkaloids from *Asteraceae*, *Boraginaceae*, *Fabaceae*; multifarious skin whitening agents; estimation of glucose¹² and steiviol glycosides; *Pelargonium graveolens*; *Serenoa repens*; and *Prunus africana*. Most importantly, the Center aided in developing an analytical approach establishing the absence of dimethylamylamine (DMAA) in authenticated *Pelargonium graveolens*. This newly developed method provided the FDA with the information required to challenge the marketing of DMAA products for lack of safety evidence on April 27, 2012. The results of this study were used to support FDA's position that DMMA found in dietary supplements sold in the U.S. (at >1 mg/g) must be synthetic. This finding led to the issuance by the agency of 10 warning letters to manufacturers and distributors of dietary supplements containing DMMA for which evidence of the safety of the product had not been submitted to FDA. The agency furthered warned the companies that synthetically-produced DMMA is not a "dietary ingredient" and, therefore, is not eligible to be used as an active ingredient in a dietary supplement.

Aim 3: Exchange technical and scientific information, analytical methods, and reference material with FDA scientists and other stakeholders.

Dr. Ikhlas Khan, Scientific Director for the project, has been in touch regularly with Dr. Daniel Fabricant (CFSAN, Director, Division of Dietary Supplement Programs), Dr. Elizabeth Calvey (CFSAN liaison), Dr. Diego Rua (CFSAN) and Dr. Robert L. Sprando, (CFSAN/OARSA, Director, Division of Toxicology) to exchange information on developed methods, reference materials availability, safety evaluations and project direction. These meetings and calls are designed to provide these individuals with an update of the NCNPR's overall program and to gain insight as to how this Center can diligently address the needs of the FDA.

Additionally, the NCNPR FDA-COE has also continued to assist the FDA's Office of Regulatory Affairs (ORA) in training inspectors for cGMP compliance for BDS's (FD340). Researchers at the NCNPR provided four one-day workshops on botanical dietary supplement authentication techniques to 135 trainees and FDA officials on May 20th, 2013, June, 19th, 2013, April 9th, 2014, May 7th, 2014 and scheduled two one-day workshops on, June 25th, 2014 and September 10th, 2014. The main training course is held in Memphis, Tennessee so that the trainees can attend a one-day excursion to the NCNPR for a combination of lectures and laboratory courses and training sessions to see what authentication techniques can be implemented for BDS's. The course covered current techniques utilized to identify botanical materials (Microscopy, Taxonomy, Macroscopy, TLC, HPLC, UPLC, GC, CE, etc.) and was presented by Dr. Khan and colleagues at the NCNPR and included two one and one half hour lab courses on Analytical Methods, Botanical Authentication, and Nomenclature. It is anticipated that this training program will continue for the foreseeable future and will provide the FDA cGMP botanical dietary supplement inspectors a valuable tool that will assist in their ability to carry out their duties.

Researchers at the NCNPR have also provided their expertise in other training offered by the FDA/ORA/DHRD. One such course was an advanced level course for analysts who are performing regulatory sample analysis using mass spectrometry techniques for identification and authentication (LB 403). Specifically, Dr. Yan-Hong Wang provided a lecture to several FDA trainees on August 28th– 29th 2013 covering the topic of how mass spectroscopy can be utilized for the authentication of botanicals. As an extension of this joint training with JIFSAN, the NCNPR sent Dr. Suman Chandra to provide four presentations at a GAP & GMP workshop aimed at supply chain management for spices and botanical ingredients for the Indian spice board (September 17th-21st, 2012, Kochi, India). Dr. Chandra covered topics such as harvesting

considerations, transportation/processing, cleaning and sanitation techniques. This workshop was then expanded into a multi-day multi-site visit for several members of the Indian spice board to provide these individuals with onsite training and lectures to provide advanced GAP and GMP techniques. This workshop, entitled "Food Safety and Supply Chain Management for Spices and Botanical Ingredients" was hosted by JIFSAN from March 25th - April 2nd then the NCNPR from April 3rd - 5th 2013. Dr. Ikhlas Khan attended the Spices Board India and All India Spice Exporters Forum, organized the World Spice Congress (WSC) in Cochin on February 16th-19th 2014. The highlight of the Congress was the Theme 'Sustainability and Food Safety' which is very relevant and crucial to the current scenario in the food sector. The business sessions planned by the Congress was led by globally renowned industry experts and addressed the topics on sustainable agriculture programs and practices based on real time experiences, infrastructural development, harmonization and simplification of standards etc. He also used this time to discuss the current collaboration between JIFSAN and the Indian Spice Board. In addition to above scientific activity, Dr. Khan and Dr. Troy Smillie participated in the 3rd Annual FDA Foods and Veterinary Medicine Science and Research Conference held in College Park, MD on August 27th -28th, 2013.

Lastly, the research effort initiated by the establishment of this Center has provided several opportunities to leverage the existing cooperative agreement so as to expand the impact and overall benefit of the existing program. This includes a recently funded NCCAM/ODS Botanical Research Center grant to explore Botanical Estrogens: Mechanisms, Dose and Target Tissues (PD: Helferich, William G., University of Illinois/PL: Khan, Ikhlas, A., Award number 5 P50 AT006268-02). Under this grant the NCNPR is providing significant quantities and populations of authenticated samples of Licorice - *Glycyrrhiza glabra* Linné var *glabra*, and Wild Yam - *Dioscorea villosa* L., for the established BRC.¹³ In addition to obtaining the outlined authenticated species for this program, we have established a substantial growing and collection program for closely related species for authentication and comparative purposes. Additionally, the NCNPR has obtained funding from the USDA/ARS for a grant entitled Discovery and Development of Natural Product-Based Insect Management Compounds for Medicinal, Veterinary and Urban Concern, award number 58-6402-1-612. The purpose of this grant is to discover and develop new, safer and more effective insect management products derived from natural phytochemical sources.

Aim 4: Collaborate with FDA scientists in research areas of mutual interest.

Over the past year the NCNPR has provided the FDA with scientific information for botanicals of concern as well as investigated several plants that have purported adverse events. Most notably we have undertaken an investigation of "chamomile" which has had reports of contact dermatitis and potential severe cases of allergic reactions in cosmetic formulations. There are two main species of "chamomile" utilized for commerce within the United States German chamomile, *Matricaria recutita* L.¹⁴ and Roman chamomile, *Anthemis nobilis*. Typically the flowering tops are used for most cosmetic formulations and these are either added as powdered material or an extract (ethanolic, supercritical or steam distilled). Working closely with scientists in the FDA/CFSAN office of cosmetics and colors, we initiated an extraction and bioassay guided fractionation of both German and Roman chamomile utilizing an LLNA screening assay for lead identification. Initial results are indicated that there is a potential sensitizer(s) within *Matricaria recutita* L. that could be causing the purported adverse events. Simultaneously, scientists at NCNPR developed two complementary *in chemico* (non-biological, non-animal) methods for identification and classification of chemical compounds as potential skin sensitizers, using either Nuclear Magnetic Resonance (NMR) spectroscopy or High Throughput Spectrophotometric methods. Further investigation including isolation, purification and *in chemico* evaluations indicated that the photo-oxidative metabolite of tonghaosu as a potential sensitizer in *Matricaria recutita* L. Scale-up, *in vivo* confirmation with LLNA screening assay have been undertaken and are still in progress.

A second project undertaken for CFSAN's office of cosmetics and colors looked at products that include an essential oil known as "Tea tree oil", which is obtained from several species of *Melaleuca* plants. One general research objective for this project is to seek further knowledge on compositional differences between commonly used species of *Melaleuca* plants (*M. alternifolia*, *M. linariifolia*, *M. viridiflora*, *M. leucadendron*, *M. dissitiflora*, etc.) in order to explore ways to address safety concerns for these species. The main safety concern about essential oils from these plant species is the potential for adverse effects on the skin, in

particular sensitization or allergic contact dermatitis (ACD). It is not fully understood which of the tea tree oil constituents is responsible for ACD. Therefore, the potential ACD active(s) in tea tree oil are currently being identified utilizing recently developed in-house NMR and HTS screening methods. Concurrently, we have developed an analytical GC/MS method to differentiate between the various species of tea tree oil that also identifies the major constituents. This newly developed method can be used to help identify potential ACD's within products.

NCNPR established two in-house preliminary *in-vivo* safety evaluations for botanicals of interest. Priority evaluation was given to botanicals that have displayed an inherent risk based on literature information or reported adverse events. Initial screens focused on two areas of concern. The first mouse model measured botanicals for their potential to induce positive reinforcement or cause aversive properties using a conditioned place preference (CPP) paradigm procedure that is commonly used to evaluate drugs for their "addictive" behavior. Over the past year the Center used this model to evaluate *Salvia divinorum*, *Mitragyna speciosa*, *Sceletium tortuosum* and fractions and pure compounds isolated from these species. Scientists at NCNPR employed the place preference/aversion paradigm to characterize the psychoactive properties of *Salvia divinorum* ext. (10, 30, 100 mg/kg), salvinin A (0.1, 0.3, 1.0 mg/kg), *Mitragyna speciosa* MeOH ext. (50, 100, 300 mg/kg), *Mitragyna speciosa* alkaloid-enriched fraction (12.5, 25, 75 mg/kg) and mitragynine (5, 10, 30 mg/kg) in rats. For *S. divinorum* the preliminary results indicated that this particular botanical did not induce abusive potential. For *M. speciosa* the results indicated that the major pharmacologically active constituent, mitragynine, has an abuse potential.¹⁵ Moreover, we have undertaken to study ADME properties of these compounds and their effect on the major efflux transporter P-glycoprotein, using *in vitro* methods. The stability of major alkaloids, mitragynine, 7-hydroxymitragynine and mitraphylline were subjected to Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF).¹⁶ All three compounds exhibited high plasma protein binding (> 90%) determined by equilibrium dialysis. Mitragynine and 7-hydroxymitragynine inhibited P-glycoprotein with EC₅₀ values of 18.2 ± 3.6 µM and 32.4 ± 1.9 µM, respectively, determined by the calcein-AM fluorescent assay, while no inhibition was seen with mitraphylline. These data suggest the possibility of a drug interaction if mitragynine and 7-hydroxymitragynine are co-administered with drugs that are P-glycoprotein substrates.

As a part of phytochemical investigation, we have isolated several known and unknown alkaloids from *S. tortuosum*. Noticeably, these marker components will assist us in authentication, identification and development of analytical methods for *S. tortuosum* and its principle alkaloid components. The majority of these findings have not yet been published; however, they will be reported shortly. Along with ADME properties and intravenous plasma pharmacokinetics, the behavioral studies associated with effects of *Sceletium tortuosum* in rats are still in progress and the results will be reported in due course.

The second *in-vivo* assay was developed to evaluate the potential hepatotoxicity's associated for botanical as measured in a mouse model where the hepatotoxicity is manifested by elevation of aminotransferases and histopathological features of acute hepatocellular necrosis and/or biliary injury. Initial *in-vivo* hepatotoxicity evaluations of EGCG,¹⁷⁻²⁰ a major component in green tea products, at high doses can lead to mild liver injury and under febrile conditions can cause severe liver injury. Currently, we are testing hepatotoxicity potential of OxyElite Pro and Black Cohosh in mice and results will be reported in due course. Both *in-vivo* models will continue to provide significant insight into the safety profile for botanicals that are of concern to public health.

Lastly, NCNPR has provided the FDA (CFSAN/OARSA) with scientific information for botanicals of concern as well as investigated several plants that are purported to have hepatotoxicity. Working closely with Dr. Sprando and his colleagues' at OARSA, we identified several sympathomimetic compounds and extracts reported to have hepatotoxic potential. Based on their usage in BDS, five whole methanolic extracts of *Astragalus membranaceus*, *Rauvolfia serpentina*, *Calea zacatechichi*, *Psoralea corylifolia*, *Adhatoda zeylanica*, *Kigelia Africana* were provided to OARSA to estimate the potential toxicity. In addition to these extracts, nine pure compounds were also provided to OARSA. Of nine pure compounds, based on practicality and other factors, four compounds, *N,N*-diethylphenethylamine, *N,N*-dimethylphenethylamine, phenethylamine, cocularine were further selected for animal studies. At this point, 5.0 Kg of cocularine was provided to OARSA and the findings will be reported in due course.

Aim 5: Coordinate scientific workshops and conferences on BDS topics of public health relevance to address high priority science and research needs.

The Center hosted the 13th Oxford International Conference on the Science of Botanicals (ICSB) that was held on April 15th – 17th, 2014, at The University of Mississippi to commemorate the 20 years of DSHEA. In addition to regulatory aspects with perspectives from government, manufacturers and trade associations; post market surveillance, risk and safety assessment, quality control and adverse event reporting (AER) for botanical dietary supplements (BDS) and natural products were discussed at 13th ICSB. This conference was co-sponsored by the Shanghai Institute of Materia Medica/CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant and Natural Products Research (GA); the Korean Society of Pharmacognosy and the Vietnam Academy of Science and Technology (VAST) the St Petersburg Institute of Pharmacy; the Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD) and the International Council on Medicinal and Aromatic Plants for Human Welfare (ICMAP) as such included representative delegations of scientists from various organizations in China, India and Europe. Due to the focused scheduling of the 13th ICSB, contributed presentations or poster submissions were not accepted. These types of presentations are reserved for the upcoming 2014 ASP/14th ICSB (<http://asp2014.org/>) which will be hosted in August 2nd - 6th 2014 Oxford, MS. This conference will cover the topics of TCM, Ayurveda, cultivation, collection, post-harvest, processing practices, identification, authentication and quality/safety assessment of botanicals including current clinical evaluations, regulatory aspects and the impact on the global dietary supplement industry. Again the proceedings from these conferences are expected to be published in *Planta Medica*.

C. Significance:

Plant collection, authentication, voucher specimens, isolation, synthesis of reference compounds and method development provide the basic tools for assessing the safety and quality of botanical products. Significant progress has been made by the NCNPR in each of these areas for several botanicals of interest over the past year. The acquired information, physical samples (plants, extracts, etc.) and phytochemical standards are freely available to researchers at the FDA for the evaluation purposes. Publications from this project have significantly contributed to the available methodological literature for the FDA and NCNPR (see references).

D. Plans:

To address the needs of the FDA on safety of BDS, Dr. Ikhlas Khan, Scientific Director for the project, would be in touch with Director, Division of Dietary Supplement Programs and liaison at CFSAN. The center will continue to exchange information on developed methods, reference materials availability, safety evaluations and project direction with CFSAN. Similar to DMAA, continual research effort will also focus on presence/absence of several alkaloids of concern for their potential safety concern due to their abuse potential, undesired adrenergic, dopaminergic receptor activities. For example, hydrastine, berberine, berberastine, hydrastinine, tetrahydroberberastine, canadine, and canalidine from *Hydrastis Canadensis*,²¹ yohimbine²² and related alkaloids from *Pausinystalia yohimbe* which is widely used as a supplement for bodybuilding and to enhance male sexual performance; Aegeline, several tetrahydroisoquinoline compounds such as boldine, reticuline and related compounds from *Aegle marmelos*.

Furthering the collaborative research effort we have established with CFSAN's office of cosmetics and colors, additional effort will continue to look at several potential areas of concern. The first being the continued exploration of products that contain "tea tree" essential oil(s) which can be derived from several species of *Melaleuca* plants. In addition to compositional differences between commonly used species of *Melaleuca* plants (*M. alternifolia*, *M. linariifolia*, *M. viridiflora*, *M. leucadendron*, etc.); the main research objective for this project will be to seek further knowledge on the sensitization potential of these essential oils using recently developed in-house in chemico methods and compare, validate the resulting data with KeratinoSens, direct peptide reactivity assay (DPRA). At the same time, we will also explore the stability, aging, isolation and identification of possible reactive intermediate(s) in these oils using the recently developed GC-MS analytical method.

Continual research effort will also focus on the two recently developed in-house *in-vivo* screen evaluating botanicals for their potential to induce positive reinforcement or cause aversive properties using the developed CPP paradigm procedure that is commonly used to evaluate drugs for “addictive” behavior and the second assay which evaluates potential hepatotoxicity associated for certain botanicals. Priority evaluation will be given to botanicals that have displayed an inherent risk based on literature information or reported adverse events. Both models will provide significant insight as to the safety profile for botanicals that are of concern to the public health.

The PI and Scientists at NCNPR will continue to work with OARSA by exchanging the scientific information on botanicals of concern with hepatotoxicity potential. For animal studies purpose, on bulk scale, three other compounds, *N,N*-diethylphenethylamine, *N,N*-dimethylphenethylamine, phenethylamines would be provided to OARSA. By working closely with OARSA, the Center will continue to provide significant insight into the authentication, validation, analytical and safety profile for BDS that are of hepatotoxic concern to public health.

To collect the information from labels which can help in determining the quantity of any given dietary ingredient, a label database will be developed. This database will assist in exposure calculations and report generation for general ingredient risk assessment. This information will be used internally as well as for public good.

Lastly, expansion of the existing repository of reference plant samples for the genera noted throughout this report will be a continuing priority for this project. New botanicals will be selected based on input received from the FDA program officer, collaborators and liaison for further studies and to evaluate their safety and quality. A fifteenth Oxford International Conference on the Science of Botanicals (ICSB) is proposed to be held on April 13th – 16th, 2015, Oxford, MS. The conference will be co-sponsored by the Shanghai Institute of Materia Medica/CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the Ministry of Indigenous Medicine, Sri Lanka; the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant and Natural Products Research (GA); the Korean Society of Pharmacognosy and the Vietnam Academy of Science and Technology (VAST) the St Petersburg Institute of Pharmacy; the Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD) and the International Council on Medicinal and Aromatic Plants for Human Welfare (ICMAP). This conference will cover the topics of TCM, Ayurveda, cultivation, collection, post-harvest, processing practices, identification, authentication and quality/safety assessment of botanicals including current clinical evaluations, regulatory aspects and the impact on the global dietary supplement industry. Again the proceedings from this conference are expected to be published in *Planta Medica*.

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Always list the PD/PI(s). In addition, list all other personnel who participated in the project during the current budget period for at least one person month or more, regardless of the source of compensation (a person month equals approximately 160 hours or 8.3% of annualized effort). Use the following abbreviated categories for describing Role on Project:

- PD/PI*
- Co-Investigator
- Faculty
- Postdoctoral (scholar, fellow, or other postdoctoral position)*
- Technician
- Staff Scientist (doctoral level)
- Statistician
- Graduate Student (research assistant)
- Non-student Research Assistant
- Undergraduate Student
- High School Student
- Consultant
- Other (please specify)

If personnel are supported by a Reentry or Diversity Supplement please indicate such after the Role on Project, using the following abbreviations: RS - Reentry Supplement; DS - Diversity Supplement.

*Commons ID required for any personnel holding this Role on Project and for all individuals supported by a Reentry or Diversity Supplement. The Commons ID will be required in the future for all individuals with a graduate student, or undergraduate role. The Commons ID is strongly encouraged, but not required, for all other Project Personnel.

Use Cal (calendar), Acad, or Summer to enter months devoted to project.

Commons ID*	Name	Degree(s)	SSN (last 4 digits)	Role on Project	DoB (MM /YY)	Cal	Acad	Summer
IKHLAS	Ikhlas Khan	Ph.D.	(b) (6)	PI	(b) (6)	5.4		
LARRYWA LKER2004	Larry Walker	Ph.D.	(b) (6)	Co-PI		1.2		
SKHAN	Shabana Khan	Ph.D.		Staff Scientist		2.76		
BAVULA	Bharathi Avula	Ph.D.		Staff Scientist		8.4		
YAN HONG	Yan Hong Wang	Ph.D.		Staff Scientist		12		
	Natasha Tehen	Ph.D.		Staff Scientist		12		
ALI	Zulfiqar Ali	Ph.D.		Staff Scientist		3		
CHITTIBOY INA	Amar Chittiboyina	Ph.D.		Staff Scientist		6		
	Gouyi Ma	Ph.D.		Staff Scientist		12		
	Ahmad Osman	Ph.D.		Staff Scientist		12		
JPZHAO	Jianping Zhao	Ph.D.		Staff Scientist		6.36		
VRAMAN	Vijayasankar Raman	Ph.D.		Postdoc		12		
	Mei Wang	Ph.D.		Postdoc		8.4		

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- Technician
- Staff Scientist (doctoral level)
- Statistician
- Graduate Student (research assistant)
- Non-student Research Assistant
- Undergraduate Student
- High School Student
- Consultant
- Other (please specify)

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Use Cal (calendar), Acad, or Summer to enter months devoted to project.

Commons ID*	Name	Degree(s)	SSN (last 4 digits)	Role on Project	DoB (MM /YY)	Cal	Acad	Summer
	Christina Avonto	Ph.D.	(b) (6)	Postdoc	(b) (6)	12		
	Amira Wanas	Ph.D.		Postdoc		6		
	Gray Dale	MA		Rschr Asst		3		
	Zhihao Zhang	Ph.D.		Postdoc		12		
	Pradip Lasonkar	Ph.D.		Postdoc		12		
	Iffat Parveen	Ph.D.		Postdoc		12		
	Vamshikrishna Manda	Ph.D.		Postdoc		12		
	Min Hye Yang	Ph.D.		Postdoc		12		
	Satyanarayanaraju Sagi	Ph.D.		Postdoc		12		
	Helaina Craig	BA		Rschr Asst		12		
	Lal Jayaratna	MSc		Rschr Asst		12		
	Steven Hopper	BFA		Technician		12		
	Jennifer Taylor			Rschr Asst		12		

MB Research Laboratories

Local Lymph Node Assay Results University of Mississippi, MB # 14-23242.26

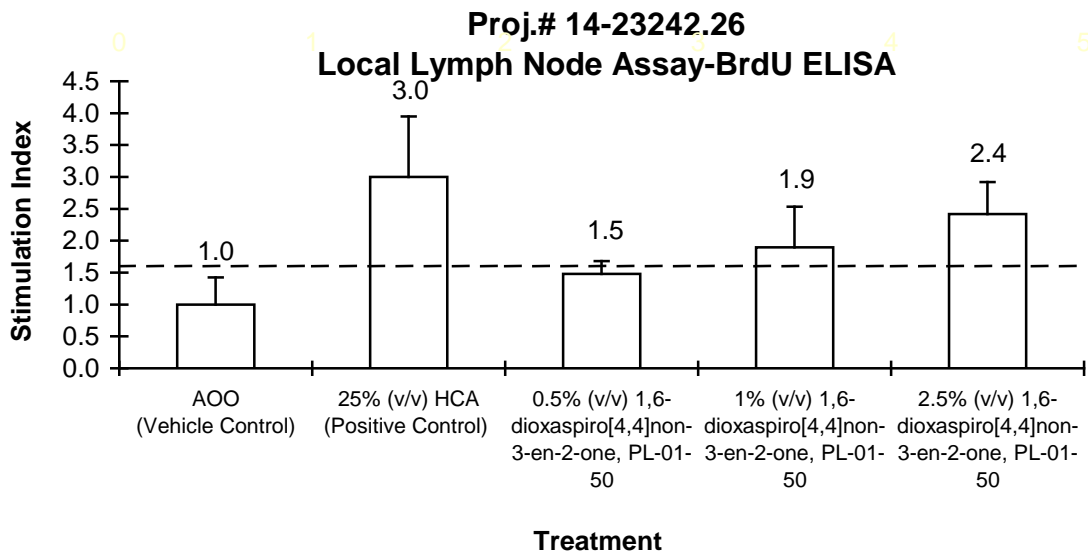
11/07/18

Reported below are the preliminary, unaudited results for the LLNA study that was conducted on 1,6-dioxaspiro[4,4]non-3-en-2-one, PL-01-50 (A.K.A. PL). This test article was negative for excessive local irritation (<25% increase in ear thickness) suggesting it is not an irritant (Table A below). Substances with a stimulation index greater than 1.6 are considered sensitizers. The stimulation index for this test article was 1.5 at 0.5%, 1.9 at 1% and 2.4 at 2.5%. Thus, the preliminary data suggest that the test article is a potential dermal sensitizer.

Table A: Irritation as Measured by Ear Swelling (% Change Day 1 to Day 6)

Acetone:Olive (4:1)	25% HCA	0.5% PL	1% PL	2.5% PL
0.0%	25.0%	0.0%	0.0%	5.0%

Table B: Stimulation Index (SI)
Local Lymph Node Assay Preliminary Results



The EC1.6, the concentration at which the stimulation index is equal to 1.6, is calculated to determine skin sensitization potency. For 1,6-dioxaspiro[4,4]non-3-en-2-one, PL-01-50, the EC1.6 = 0.65%.

✓ *List of current 26 allergens*



- Amyl Cinnamal
- Amylcinnamyl Alcohol
- Anise Alcohol
- Benzyl Alcohol
- Benzyl Benzoate
- Benzyl Cinnamate
- Benzyl Salicylate
- Butylphenyl Methylpropional
- Cinnamyl Alcohol
- Citral
- Citronellol
- Coumarin
- Eugenol
- Farnesol
- Geraniol
- Hexyl Cinnamal
- Hydroxyisohexyl 3-cyclohexene Carboxaldehyde
- Hydroxycitronellal
- Isoeugenol
- Alpha-isomethyl Ionone
- Limonene
- Linalool
- Methyl 2-Octynoate
- Oak Moss Extract
- TreeMoss Extract
- Cinnamal

Quantitative Analysis of the 26 Allergens for Cosmetic Labeling
in Fragrance Raw Materials and Perfume Oils

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The adoption of the 7th amendment of the European Cosmetic Directive 76/768/EEC requires any cosmetic product containing any of 26 raw materials identified by the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers as likely to cause a contact allergy when present above certain trigger levels to be declared on the package label. Of these 26, 24 are volatile and can be analyzed by GC. This paper describes a method for the quantitative analysis of these volatile raw materials in perfume ingredients as well as complex perfume compositions. The method uses sequential dual-column GC-MS analysis. The full-scan data acquired minimize the false-positive and false-negative identifications that can be observed with alternate methods based on data acquired in the SIM mode. For each sample, allergen levels are determined on both columns sequentially, leading to two numerical results for each allergen. Quantification limits for each allergen in a perfume mixture based on the analysis of a standard are <4 mg/kg. This is well below the level that would trigger label declaration on the consumer good. Calibration curves for all allergens are linear ($r > 0.999$) and stable for multiple days. Studies on perfumes spiked with multiple allergens at 30, 50, and 70 mg/kg show recoveries close to nominal values.

KEYWORDS: Perfumes; fragrances; allergens; analytical method

INTRODUCTION

In 2003 Directive 2003/15/EC, the 7th amendment of the European Cosmetic Directive 76/768/EEC, was published (1). This directive requires that any cosmetic product containing any of 26 raw materials above certain trigger levels must declare these ingredients on the label in descending order of weight. An analytical method for markers in oak moss is reported elsewhere (7). Therefore, it was decided not to include tree moss and oak moss in the development of this method. Labeling, using International Nomenclature Cosmetic Ingredient (INCI) names, is required when the level of the individual ingredient exceeds 10 mg/kg in a product intended to remain on the skin or 100 mg/kg in a product to be rinsed off of the skin.

As a result of this directive, it became necessary to develop analytical methods enabling identification and quantification of low levels of these ingredients in the presence of highly complex mixtures, such as fragrances and their raw materials. As with all methods, situations can occur in which the results are negatively influenced by matrix effects. These include coeluting components or closely eluting components. In such situations, false positives, false negatives, or incorrect quantification may result. In response to these issues, we have developed a method in our laboratory that has clear advantages in terms of preventing false positives and false negatives as well as minimizing

inaccurate quantification resulting from coelution or other chromatographic disturbances. This paper describes a methodology that we have developed and are now using regularly in our quality control laboratories.

MATERIALS AND METHODS

GC-MS Analysis. GC-MS analysis was carried out on a Shimadzu QP2010 mass spectrometer coupled to a Shimadzu GC-2010 gas chromatograph equipped with a CTC autosampler. The system was equipped with two split/splitless injectors. Each injector was connected to a column of different polarity. Both columns are connected to the MS interface using a dual-hole ferrule. Details of the columns and conditions used are shown in Table 1.

Standards Preparation. Each of the 24 ingredients in this study are regularly used fragrance ingredients and are shown in Table 2. The purity of each ingredient was verified prior to use. Each was above 95.0% purity as determined by GC-FID area percent measurements. For purposes of this study, the quantities of cis and trans isomers were added together. Exceptions were 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one, which was 89.7% pure, and hydroxymethylpentyl-cyclohexenecarboxaldehyde, which consisted of 72% of the 4-isomer and 27% of the 3-isomer. A special note should be made on *d*-limonene. The EC directive (1) refers to *d*-limonene (CAS Registry No. 5989-27-5). The method described in this paper cannot distinguish between *d*-limonene and *l*-limonene. For reasons of consistency reference will be made to *d*-limonene throughout the paper.

Standards were stored as individual chemicals in a freezer (-25 °C) for a maximum of 3 months. Each month, a mixture (mixture A) was prepared from these pure ingredients (~ 1 g of each ingredient) and stored in a freezer (-25 °C). A calibration stock solution was made

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Table 1. Materials, Equipment, and Settings Used for Allergen Analysis

GC-MS system	Shimadzu QP2010 connected to a Shimadzu GC-2010 equipped with a CTC autosampler
GC column	Varian CpSil 5 CB 50 m × 0.25 mm × 0.25 μm or SGE Solgel1 60 m × 0.25 mm × 0.25 μm (both bonded polydimethylsiloxane)
internal standards	Varian CpWax 52 CB 50m × 0.25 mm × 0.20 μm 2,3-dichlorotoluene, CAS [32768-54-0] 1,4-dibromobenzene, CAS [106-37-6] acetone, GC grade, CAS [67-64-1]
solvent	1 μL
injection volume	250 °C
injection temperature	mode
mode	split, 1:10
column pressure	170 kPa, constant pressure, both columns
carrier gas	helium
temperature program	column 1, 50 °C, 1 min; ramped at 12 °C/min to 250 °C, 11 min; cooled at -40 °C/min to 120 °C, 3 min column 2, 120 °C, 3 min, ramped at 4 °C/min to 216 °C, 0 min; ramped at 10 °C/min to 250 °C, 13 min
interface temperature	250 °C
source temperature	200 °C
MS parameters	full scan, <i>m/z</i> 30–372, scan speed of 2000 amu/s
calibration	eight levels from 2 to 60 mg/kg

Table 2. Fragrance Ingredients and Their CAS Registry Numbers^a

name	CAS Registry No.
amylcinnamyl alcohol	101-85-9
amyl cinnamal	122-40-7
anisyl alcohol	105-13-5
benzyl alcohol	100-51-6
benzyl benzoate	120-51-4
benzyl cinnamate	103-41-3
benzyl salicylate	118-58-1
cinnamyl alcohol	104-54-1
cinnamal	104-55-2
citral (mixture of neral and geranial)	5392-40-5
citronellol	106-22-9
coumarin	91-64-5
eugenol	97-53-0
farnesol (main isomers, <i>ZE</i> and <i>EE</i>)	4602-84-0
geraniol	106-24-1
hexyl cinnamic aldehyde	101-86-0
hydroxy-citronellal	107-75-5
isoeugenol	97-54-1
2-(4- <i>tert</i> -butylbenzyl) propionaldehyde	80-54-6
<i>d</i> -limonene	5989-27-5
linalool	78-70-6
hydroxy-methylpentyl-cyclohexenecarboxaldehyde	31906-04-4
methyl heptin carbonate	111-12-6
3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)- -buten-2-one	127-51-5

^a CAS Registry No. provided by the author.

weekly from mixture A by diluting 0.1 g of mixture A to 20 g with acetone (~208 mg/kg each ingredient).

The internal standard solution was prepared bimonthly by diluting 2,3-dichlorotoluene and 1,4-dibromobenzene in toluene (~1000–1500 mg/L). Each calibration standard was prepared by mixing the appropriate amount (weight) of the stock solution with 100 μL of the internal standard solution. This mixture was then further diluted with acetone to 10 g. This way, calibration standards of approximately 2, 5, 10, 20, 30, 40, 50, and 60 mg/kg of each ingredient were prepared. For quantification, 2,3-dichlorotoluene was used. In those cases when this internal standard coelutes with an unknown containing the same ion used as the quantifier for 2,3-dichlorotoluene, 1,4-dibromobenzene may be used as an alternative.

Sample Preparation. Perfume samples were prepared by adding 100 μL of the internal standard solution to 0.5 g of the neat perfume oil. This mixture was diluted to 10 g with acetone. Raw material samples were prepared by adding 100 μL of the internal standard solution to 0.1 g of the neat raw material. This mixture was diluted to 10 g with acetone. The applied dilution of both perfume and raw material samples in acetone is done to diminish overload in the GC-MS system.

Table 3. Quantifier and Qualifier Ions for Allergen Analysis

name	quantifier; qualifier ions (CpSil 5 CB or Solgel 1 or CpWax 52 CB)
amylcinnamyl alcohol	91; 115, 133, 204
amyl cinnamal	145; 115, 129, 202
anisyl alcohol	138; 109, 121, 137
benzyl alcohol	108; 79, 107
benzyl benzoate	105; 91, 212
benzyl cinnamate	131; 91, 192
benzyl salicylate	91; 65, 228
cinnamyl alcohol	134; 92, 105, 115
cinnamal	131; 77, 103, 132
citral	
neral	69; 84, 94, 109
geranial	69; 84, 94, 109
citronellol	81; 95, 123
coumarin	118; 89, 90, 146
eugenol	164; 103, 149
farnesol	
ZE isomer	69; 81, 93
EE isomer	93; 69, 81
geraniol	93; 69, 123
hexylcinnamic aldehyde	145; 117, 129
hydroxycitronellal	59; 71, 81, 96
isoeugenol	164; 103, 149
2-(4- <i>tert</i> -butylbenzyl) propionaldehyde	189; 117, 131, 147
<i>d</i> -limonene	68; 67, 93, 121
linalool	71; 80, 93, 121
hydroxy-methylpentyl-cyclohexene- carboxaldehyde	136; 93, 149, 192
methyl heptin carbonate	123; 67, 79, 95
3-methyl-4-(2,6,6-trimethyl-2-cyclo- hexen-1-yl)-3-buten-2-one	150; 107, 135
2,3-dichlorotoluene (IS)	125; 127, 160, 162
1,4-dibromobenzene (IS)	236; 234, 238

Analysis. Samples were analyzed sequentially on each column with full-scan mass spectral acquisition. The use of full-scan spectra allows for positive identification using a library search. Qualifier and quantifier ions were selected for each target compound as shown in **Table 3**. Response factors are calculated from the quantifier ion signal for the internal standard and target components. For quantification of target components, the above-determined response factor is applied on its quantifier ion signal. Prior to evaluation, all data are subjected to a Savitzky–Golay filter using 11 data points.

RESULTS AND DISCUSSION

Potential Chromatographic and Spectral Issues. A single-column GC run will separate most of the components of a

perfume, but in a substantial number of cases coelution will occur. For correct quantification and identification this does not have to be a problem as long as the GC detector can provide unique information for the components of interest. GC-MS electron impact (EI) generated spectra provide such additional information. However, even with this second dimension, it is often not possible to separate all components of interest. Coeluting components may give rise to mixed spectra.

The analytical method published by the International Fragrance Association (IFRA) (2), which is based on GC-MS, uses a combination of retention time and the ratio of selected ions acquired in the SIM mode for positive identification. Quantification is based on a single ion. The method uses multiple SIM windows with mostly three ions and a time width as small as 0.1 min. This method works well with perfume compositions for which no coelution occurs with a component having the same quantifier and qualifier ion of the target component and for which retention times are constant. Sometimes, however, these conditions are not met. For example, a large nonrelated peak that elutes in front of the SIM window for a potential allergen may cause the allergen to shift out of the SIM window, resulting in a false negative. The coelution of an ingredient with ions identical to those used for the identification and quantification of an allergen may also cause problems. First, the coelution may cause the ion ratios of quantifier and qualifier ions to be within set parameters, leading to a false-positive identification. Second, the ion ratio may remain within the parameters set for positive identification, but lead to incorrect quantification. Third, ratios of quantifier and qualifier ions may be outside set limits, leading to false-negative identification.

Multiple alternative approaches for overcoming the above-described situations exist. To prevent false negatives as a result of retention time shift, a wider SIM window with more ions can be used. However, when the equipment is running in SIM, if the component causing the retention time shift does not have any of the ions in the SIM window, the identification relies more heavily on the correct ion ratios because the retention times do not match. A full-scan analysis to verify the retention time shift may be necessary. This approach will not solve the problem of a true coelution of an allergen with a component that contains one of the qualifier or quantifier ions used in the SIM window.

In cases when the ratios of the quantifier and qualifier ions do not match the set ratio criteria, but a peak is found in the SIM window, a full scan can be run to prove the presence or absence of the allergen. This requires an additional analysis and does not solve all coelution problems. It may, however, reveal that one of the ions used in the previous SIM analysis is unique and can be used for quantification instead of qualification. Still, this requires recalibration and reanalysis.

Another way to handle a questionable identification is spiking of the sample with the suspected allergen. This requires a reasonable estimate of the actual allergen level, followed by spiking, and then an additional time-consuming analysis. Other alternatives include GC-MS-TOF (4) using mass spectral deconvolution to extract coeluting peaks from complex chromatograms. GC-MS-CI and comprehensive GC have also been reported as alternatives (5, 6).

Advantages of the Current Method. The method developed in our laboratory takes advantage of the benefits of full-scan acquisition and dual-column analysis. The sample is analyzed sequentially on columns of different polarities in full-scan mode. This way, the identical sample is analyzed twice. This setup has a number of advantages for the complex chromatographic situations described above. In the case of coelution, the second

Table 4. Quantification Limits of Allergens in a Perfume Oil Determined Using a Standard Mixture of Allergens

name	quantification limit (mg/kg)	
	CpSil 5 CB or Solgel 1	CpWax 52 CB
amylcinnamyl alcohol	2.65	1.77
amyl cinnamal	1.35	2.00
anisyl alcohol	1.42	1.04
benzyl alcohol	0.44	0.32
benzyl benzoate	0.61	0.55
benzyl cinnamate	3.27	2.60
benzyl salicylate	0.82	0.38
cinnamyl alcohol	1.71	1.11
cinnamal	1.11	0.34
citral		
neral	0.17	0.38
geranial	0.25	0.26
citronellol	0.85	1.25
coumarin	1.18	0.51
eugenol	0.56	0.62
farnesol		
ZE isomer	2.03	0.82
EE isomer	1.49	0.96
geraniol	1.93	1.07
hexylcinnamic aldehyde	1.99	0.75
hydroxycitronellal	1.63	0.76
isoeugenol	1.28	0.63
2-(4-tert-butylbenzyl) propionaldehyde	0.27	0.23
α -limonene	0.23	0.42
linalool	0.49	0.71
hydroxymethylpentylcyclohexene carboxaldehyde	2.16	1.90
methyl heptin carbonate	0.61	0.35
3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	0.55	0.38

column chromatogram is directly available for positive identification. The full-scan spectra allow for positive identification using a library search. Qualifier ions are used to prescreen the chromatogram for target components. Potential retention time shifts have no negative effect because the complete chromatogram is recorded in full scan. Potential coelutions on both columns can easily be overcome by choosing any suitable quantifier ion. Because all spectra are recorded in full scan, no additional acquisition is needed.

Examination of **Table 3** shows that the most abundant ion is not always chosen as the quantifier. Ions are chosen such that the chances for coelution with isobaric ions are minimized. Dichlorotoluene was used as the primary internal standard for all target components. The calibration curves for all target compounds were found to be linear throughout the calibration range (correlation coefficient $r > 0.999$) and stable for multiple days.

Quantification Limits. **Table 4** shows the quantification limit for each target compound in a fragrance oil determined using a sample containing each allergen at a level of ~ 0.23 mg/kg. It was determined by multiplying the noise of the quantifier ion signal on either side of the target component by 10. Because we used a standard to determine the quantification limit, matrix effects that can occur in raw materials and perfumes were minimized. Such effects can negatively influence the quantification limit. An experiment was done to determine if the calibration of the system would change over time. First, the calibration curves were constructed from the standards. Next, the system was used for other purposes for two full days, analyzing 34 other samples (perfumes and raw materials). After that, calibration standard mixtures of approximately 2 and 6 mg/kg were recorded 10 times. These levels correspond to approximately 40 and 120 mg/kg, respectively, for each allergen in the perfume oil. **Table 5** shows the interval of confidence ($n = 10$, 95%) for each target component for both of these levels expressed as equivalent levels in perfume oils.

Table 5. Interval of Confidence for Allergens Based on 10 Repetitive Analyses of Standards

name	confidence intervals ($n = 10$, 95%) (mg/kg) in perfume oil					
	level 1			level 2		
	nominal	Solgel 1	CpWax	nominal	Solgel 1	CpWax
amylcinnamyl alcohol	31.1	30.3–31.9	31.3–32.4	120	119–124	119–122
amyl cinnamal	33.2	33.4–35.4	32.8–34.0	128	126–134	122–130
anisyl alcohol	32.5	31.1–32.2	32.7–33.4	126	124–127	127–130
benzyl alcohol	32.5	31.7–32.2	32.3–33.1	126	124–126	125–127
benzyl benzoate	32.6	34.8–36.1	33.3–34.0	126	131–135	127–131
benzyl cinnamate	32.7	31.9–33.6	32.2–33.4	126	126–131	130–137
benzyl salicylate	32.6	31.7–32.4	32.0–32.8	126	121–126	123–127
cinnamyl alcohol	32.1	30.5–31.8	31.5–33.1	124	120–124	122–125
cinnamal	32.7	32.0–32.8	32.3–32.8	126	119–127	118–124
citral						
neral	13.6	13.4–13.9	13.5–14.1	53	52–54	52–54
geranial	17.9	17.1–17.7	17.5–18.2	69	68–69	68–69
citronellol	32.4	31.4–32.4	32.2–33.0	125	123–125	125–127
coumarin	32.4	32.3–32.8	32.3–33.1	125	127–129	124–128
eugenol	32.8	32.2–33.1	31.3–32.5	127	127–129	123–127
farnesol						
ZE isomer	15.6	14.2–15.1	15.2–15.8	60	57–59	58–60
EE isomer	15.8	15.4–16.3	15.7–16.3	61	59–61	60–63
geraniol	32.1	31.9–32.8	32.0–33.1	124	121–125	121–125
hexylcinnamic aldehyde	32.5	33.3–34.6	32.8–33.7	126	126–133	124–128
hydroxycitronellal	32.4	31.9–32.6	31.7–32.7	125	125–127	124–127
isoeugenol	32.5	31.5–32.3	31.3–32.0	125	122–128	122–126
2-(4- <i>tert</i> -butylbenzyl) propionaldehyde	32.2	33.5–34.1	32.1–32.9	125	126–129	124–127
<i>d</i> -limonene	32.1	32.2–32.6	32.3–33.0	124	123–126	124–126
linalool	31.7	31.4–31.9	31.4–32.0	123	121–123	123–124
hydroxymethylpentylcyclohexene carboxaldehyde	32.6	32.7–34.0	32.5–34.5	126	129–132	124–129
methyl heptin carbonate	32.4	31.5–32.5	32.3–33.1	125	124–125	125–129
3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	29.2	30.0–30.7	28.7–29.3	113	113–117	111–114

Table 6. Quantitative Results for Spiked Perfume Samples

(A) Samples Spiked at 30 mg/kg						
component	nonspiked sample		30 mg/kg spike level		nominal (mg/kg)	
	Solgel (mg/kg)	CPWax (mg/kg)	Solgel (mg/kg)	CPWax (mg/kg)		
benzyl alcohol			29	34		32
benzyl cinnamate			32	30		31
benzyl salicylate			coelute	35		34
cinnamal			coelute	30		32
citronellol			coelute	30		31
eugenol			coelute	30		32
2-(4- <i>tert</i> -butylbenzyl) propionaldehyde			31	30		32
<i>d</i> -limonene	10	9	37	37		30
linalool			30	32		30
hydroxy-methylpentyl-cyclohexene carboxaldehyde			coelute	33		31
(B) Samples Spiked at 50 and 70 mg/kg						
component	50 mg/kg spike level		nominal (mg/kg)	70 mg/kg spike level		nominal (mg/kg)
	Solgel (mg/kg)	CPWax (mg/kg)		Solgel (mg/kg)	CPWax (mg/kg)	
benzyl alcohol	50	54	54	74	76	76
benzyl cinnamate	52	53	53	72	69	74
benzyl salicylate	coelute	55	56	coelute	80	79
cinnamal	coelute	51	53	coelute	70	75
citronellol	coelute	53	52	coelute	74	74
eugenol	coelute	50	53	coelute	73	74
2-(4- <i>tert</i> -butylbenzyl) propionaldehyde	49	54	53	69	74	74
<i>d</i> -limonene	57	57	50	75	76	71
linalool	53	50	51	72	72	72
hydroxymethylpentylcyclohexene carboxaldehyde	coelute	52	52	coelute	76	73

Full-Scan Quantification Results. To further demonstrate the quantitative performance of the extracted ion full-scan method, a perfume free from the 26 allergens was spiked with 10 of them at nominal levels of 30, 50, and 70 mg/kg. The samples were analyzed using a Solgel 1 column and a CpWax 52CB column as specified above.

Analysis of the nonspiked sample revealed the presence of 9–10 mg/kg of *d*-limonene. Levels of *d*-Limonene in the spiked samples are not corrected for this background level. As the data

in **Table 6** show, the average deviation from nominal values is very small. Furthermore, where coelution did occur, no recalibration for a different target ion is needed because quantification from the second column can be used.

The data presented show clearly that a full-scan method for the identification and quantification of the 24 volatile potential allergens identified for cosmetic labeling in perfume oils and perfume ingredients has excellent characteristics. The chance for false positives and false negatives is low, and the quality of

the quantitative data is high. In cases of coelution, reanalysis is not needed because a second chromatogram is readily available. If this fails to solve the coelution problem, the full-scan data allow for the immediate search of a unique ion. Because the calibration data are also recorded full scan, no rerun of calibration standards is needed. The new calibration can immediately be done on the existing calibration data. The excellent quantitative performance of the method is demonstrated by the quantification limits, interval of confidence data, and recovery results from spiked samples.

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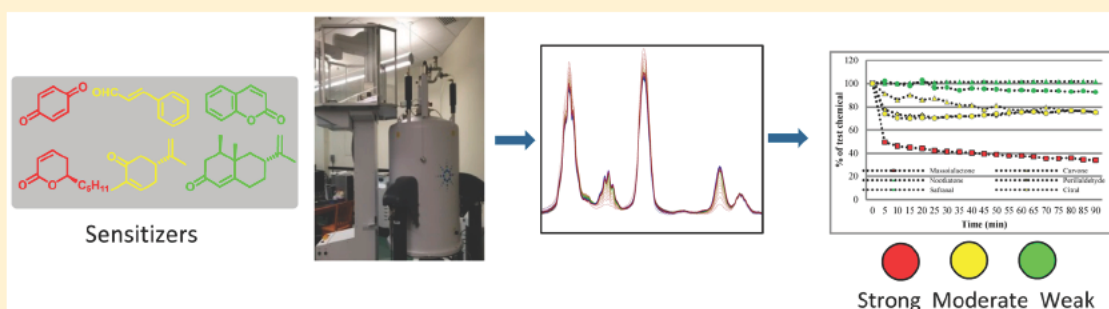
Alternative Testing Methods for Skin Sensitization: NMR Spectroscopy for Probing the Reactivity and Classification of Potential Skin Sensitizers

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Supporting Information



ABSTRACT: Evaluating consumer products for potentially harmful side effects of chemical ingredients is important for the protection of both the consumer and those involved in the manufacturing process. In order to assess the risk potential of chemicals, regulatory agencies have encouraged the development of several *in silico*, *in vitro*, and *in chemico* methods as alternatives to eliminate or minimize the use of animals. To add structural information to the existing *in chemico* methods, an NMR based method is proposed for probing the reactivity and classification of the potential electrophiles (E) using a model thiol, DCYA, as a nucleophile. The major advantage of the NMR method is the quantitation of the actual adduct, DCYA E. The degree of reaction is here provided as a direct measurement of adduct formation and/or electrophile depletion, in contrast to other *in chemico* assays, e.g., ADRA and DPRA, where the reactivity is inferred from the quantification of the test nucleophile depletion. Moreover, the developed NMR method should serve as a qualitative and quantitative tool in understanding the site of reaction and other structural information associated with test sensitizer. This is particularly valuable and advantageous over methods encouraged by regulatory agencies, which merely provide quantification of the reaction but lack any structural information. Several compounds with multiple reaction sites were successfully tested with the proposed NMR method. Otherwise, these compounds have proven to be a challenge to identify and classify using existing alternative methods.

INTRODUCTION

Skin sensitization represents the biological end point used to classify a more complex immunopathology known as allergic contact dermatitis (ACD). ACD is a common occupational, environmental health problem,¹ and it is characterized by two major processes: sensitization and elicitation. In the first step, contact with a potential chemical allergen (hapten) will stimulate the production of antigens that are responsible for the recruit and activation of T lymphocytes. Further contact with the same hapten (elicitation) can result in the production of a vigorous immunoreaction, which often results in rash, redness, and skin lesions, with severe outcomes in the worst cases.² For toxicological purposes, the sensitization potential of a chemical compound represents the event to be identified, whereas the elicitation phase is the clinical manifestation of the pathology and may occur even years after the sensitization step. A potential

sensitizer can interact with its biological target through covalent binding to form an antigenic complex. Reactive amino acids, including cysteine, lysine, and, to a lesser extent, arginine, histidine, methionine, and tyrosine residues, have been suggested to play a key role in skin sensitization.³ Although no specific target has yet been clearly identified, several chemical features, such as low molecular weight, lipophilicity, and chemical reactivity are considered to be essential for a potential sensitizer.⁴ At present, approximately 4000 low molecular weight allergens have been identified. The majority of these compounds are lipophilic and have been classified according to their mechanistic domains.⁵ Figure 1 summarizes the most common mechanistic domains identified, and many of the strong and moderate

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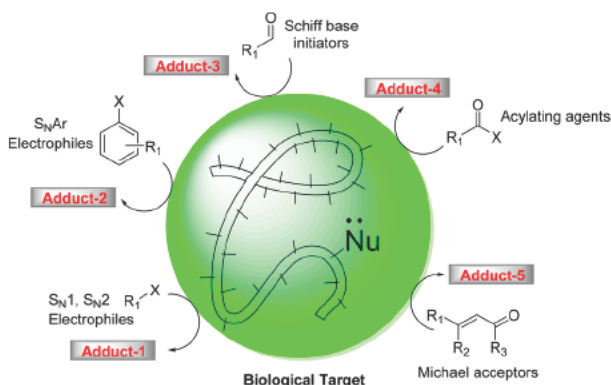


Figure 1. Schematic representation of plausible chemical reactions involved in haptation processes.

sensitizers have been classified as electrophilic α,β unsaturated carbonyl compounds.⁶

Laboratory animals have been widely used^{7–10} in the past for skin sensitization risk assessment, but for many years, regulatory agencies have encouraged the development of alternative techniques to eliminate or minimize the use of animals.

Several *in silico*, *in vitro*, and *in chemico* alternative methods have been developed to address possible adverse outcomes by incorporating the 3Rs (replacement, reduction, and refinement) concept. While *in silico* methods (QSAR Toolbox, Derek,¹¹ TIMES SS,¹² and ToxTree¹³) are based on structure–activity relationships, *in vitro* assays model the early events of the skin sensitization process. Nrf2 based assays^{14,15} (KeratinSens, LuSens) analyze the induction of the cellular antioxidant pathway, and dendritic cell based assays (MUSST,¹⁶ hCLAT¹⁷) measure DC maturation markers (CD86, CD54). On the other hand, chemical reactivity assays (DPRA,^{18–20} GSH,²¹ ADRA²²) reflect the haptation mechanism associated with skin sensitization. Some of these methods were examined by the European Centre for the Validation of Alternative Methods (ECVAM); the DPRA and the ARE Nrf2 luciferase methods have been recently adopted by the Organization for Economic Co operation and Development (OECD) for testing health effects of chemicals.^{23,24}

Existing *in chemico* methods have been continuously refined to address potential pitfalls, such as solubility and activation problems. To improve the solubility of lipophilic allergens in aqueous solutions, experiments with microemulsion systems²⁵ were suggested. Preincubation of prohaptens with peroxidases has been proposed to mimic the metabolic activation to reactive intermediates.^{26,27}

In silico approaches are computational methods that rely on the presence of structural alerts. Predictive methods may fail to identify sensitizers that possess undefined mechanistic domains or the presence of pre or pro sensitizers. Moreover, assessment of test material with more than one component has been proved to be an elusive task with existing methods except for the KeratinSens assay. Recently, Natsch and co workers²⁸ evaluated the applicability of the KeratinSens assay for plant extracts used in the cosmetic field as a proof of concept. However, there were limitations for an extract with high cytotoxicity, in which case detection of the artificially spiked sensitizers proved to be difficult. The same limitation may be extrapolated to all cell based assays.

Overall, each of the above non animal alternative methods may be unable to completely predict the skin sensitization potential of a broad range of chemicals. The majority of state of the art *in chemico* methods (standalone or in combination with

in silico predictions) would be able to provide the critical information on how much or how fast the reaction occurs for a given test compound's sensitization potential. However, important questions are still unanswered about where the reaction occurs and what the chemical structures of the resulting adducts are. To address these critical queries and to add additional knowledge to the current testing protocols, we demonstrate herein an NMR based method for probing the reactivity and classification of potential skin sensitizers (PCT application filed, PCT/US15/38142).

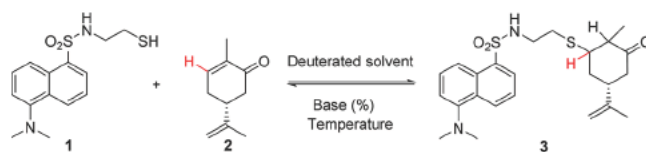
EXPERIMENTAL PROCEDURES

Chemicals. Dansyl chloride, cystamine hydrochloride, cinnamaldehyde, *p* benzoquinone, *p* hydroquinone, 3 hydroxytyrosol, coumarin, curcumin, ethyl acrylate, citral, saffranal, parthenolide, costunolide, alantolactone, 1,5 diazabicyclo[4.3.0]non 5 ene (DBN), and 2,5 dimethylfuran were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Massoia lactone, *trans* 2 pentenal, (–) carvone, (–) perillaldehyde, and nootkatone were kindly donated by Citrus and Allied Essences Ltd. (Lake Success, NY, USA). All deuterated solvents, chloroform *d*₃, acetonitrile *d*₃, and dimethyl sulfoxide *d*₆ were purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA). Dansyl cysteamine (DCYA) was synthesized by following a slightly modified version of a reported procedure using commercially available dansyl chloride and cystamine hydrochloride (Synthesis section).

NMR Spectroscopy. Reactions were followed by monodimensional ¹H NMR on an Agilent 500 MHz spectrometer (number of scans = 4; gain = 30; spectral size = 74 850; δ –1 to 14 ppm; acquisition time = 5 s; spectral width = 7485.0 Hz; FID resolution = 0.20 Hz; acquisition size = 19 461; delay time = 20 s; pulse width = 30°; temperature = 25 °C). Chemical shifts are reported in ppm relative to the ¹H residual signal of the solvent peak (CDCl₃, 7.26; DMSO *d*₆, 2.50; and acetonitrile *d*₃, 1.94). 2,5 Dimethylfuran was used as the internal standard. When necessary, characterization of the product(s) formed during the reaction(s) was performed by homonuclear ¹H connectivities using 2D COSY experiments. One bond heteronuclear ¹H–¹³C connectivities were determined using gradient HMQC experiments in which the interpulse evolution period was optimized to 3.45 μ s. Two and three bond ¹H–¹³C connectivities were determined by gradient HMBC experiments in which the evolution period for long range ¹H–¹³C coupling constants was optimized for a ²³J_{CH} of 8 Hz. Through space ¹H connectivities were determined using a NOESY experiment with a mixing time of 0.450 s. Chemical shifts were compared with those calculated using ACD/C+H NMR Predictors and DB software [release 2012 (build 60488, 08 Nov 2012), ACD/Laboratories, Toronto, Canada].

Synthesis. *N,N'*-(Disulfanediylbis(ethane-2,1-diyl))bis(5-(dimethylamino)naphthalene-1-sulfonamide) (DCYA Disulfide). In a 250 mL round bottomed flask, dansyl chloride (0.88 g, 3.25 mmol) was dissolved in a solution of 90 mL of acetone and 3 mL of water. A solution of cystamine dihydrochloride (0.36 g, 1.62 mmol, 0.5 equiv) in 21 mL of 0.1 M aqueous NaHCO₃ was added in portions. The solution was maintained to pH 7.5 by addition of aqueous 0.5 M sodium hydroxide. After 90 min at room temperature, the reaction mixture was diluted with 100 mL of chloroform, and the solution was washed four times with aqueous sodium bicarbonate, followed by water. The organic layer was dried over anhydrous MgSO₄ concentrated, and purified by

Scheme 1. NMR Experiment with DCYA (1) and L Carvone (2) with and without Addition of a Base^a



^aThe proton in red corresponds to the signal monitored by NMR.

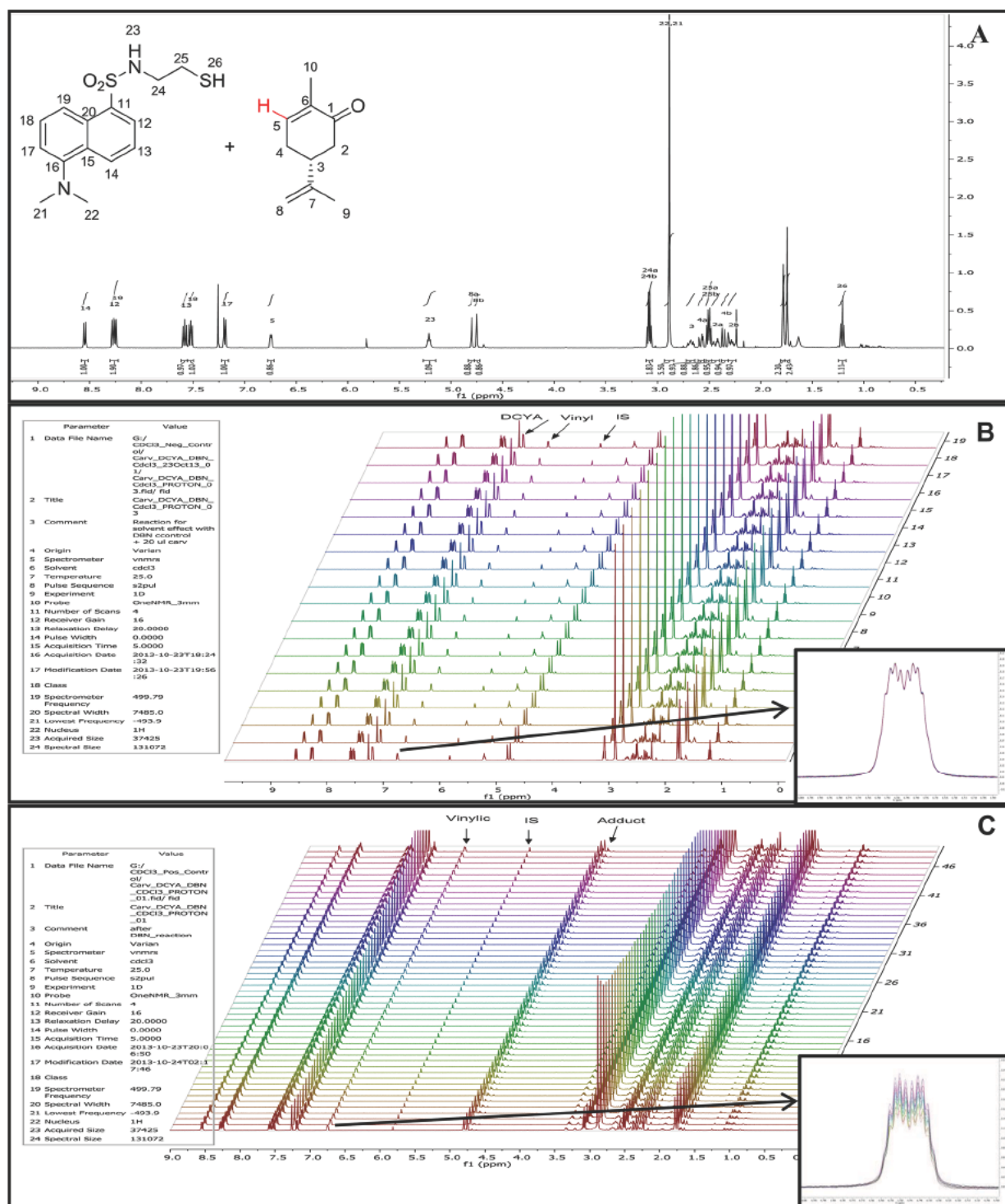


Figure 2. (A) ^1H NMR spectra of a 1:1 mixture of DCYA and l-carvone before addition of DBN; (B) array of 19 ^1H NMR spectra (500 MHz) of both DCYA and carvone collected for 90 min prior to the addition of a catalytic amount of DBN in CDCl_3 ; (C) array of 45 ^1H NMR spectra collected after the addition of DBN within a 5 h period. Expansion between δ 6.80 and 6.70 ppm for the concentration change of the vinylic proton is shown in the insets.

column chromatography to yield 0.9 g of disulfide as a fluffy, crisp yellow solid (1.45 mmol, 90%), TLC R_f = 0.25, 30% acetone in hexanes, mp 71–72 °C. IR (cm^{-1}): 3280, 2942, 1574, 1454, 1408, 1311, 1140, 1063, 948, 789; ^1H NMR: (500 MHz, CDCl_3) δ 8.54 (d, J = 8.5 Hz, 1H), 8.27–8.20 (m, 2H), 7.53 (dt, J = 8.6, 7.4 Hz, 2H), 7.17 (d, J = 7.6 Hz, 1H), 5.24 (t, J = 6.2 Hz, 1H), 3.09 (q, J = 6.3 Hz, 2H), 2.88 (s, 6H), 2.48 (t, J = 6.3 Hz, 2H); ^{13}C NMR: (125 MHz, CDCl_3) δ 152.2, 134.5, 130.8, 130.0, 129.8, 129.6, 128.7, 123.3, 118.7, 115.4, 45.6, 41.7, 37.9; ESI MS ($\text{C}_{28}\text{H}_{34}\text{N}_4\text{O}_4\text{S}_4$): m/z 619.2 [$M + \text{H}$] $^+$.

5-(Dimethylamino)-N-(2-mercaptoethyl)naphthalene-1-sulfonamide (DCYA). In a 50 mL round bottomed flask, the disulfide compound (0.32 g, 0.524 mmol) was dissolved in THF (18 mL) and water (2 mL) and cooled to 0 °C, and sodium borohydride (0.20 g, 5.29 mmol) was added in small portions. After 4 h, the solvent was evaporated, and the residual was diluted with water and extracted with diethyl ether (3 \times 10 mL). The combined organic layers were dried over anhydrous MgSO_4 , concentrated, and purified to yield DCYA thiol as greenish yellow fluffy solid (0.13 g, 83%). (TLC 30% EA in hexane,

$R_f = 0.40$). IR (cm^{-1}): 3256, 2949, 2828, 1577, 1428, 1402, 1301, 1137, 1090, 1078, 940, 845, 786; ^1H NMR (500 MHz, CDCl_3) δ 8.56 (d, $J = 8.5$ Hz, 1H), 8.28 (d, $J = 8.6$ Hz, 1H), 8.25 (dd, $J = 7.4, 1.1$ Hz, 1H), 7.58 (t, $J = 7.6$ Hz, 1H), 7.53 (dd, $J = 8.4, 7.4$ Hz, 1H), 7.20 (d, $J = 7.6$ Hz, 1H), 5.18 (t, $J = 6.3$ Hz, 1H), 3.08 (q, $J = 6.4$ Hz, 2H), 2.89 (s, 6H), 2.51 (dt, $J = 8.7, 6.4$ Hz, 2H), 1.21 (t, $J = 8.7$ Hz, 1H); ^{13}C NMR (125 MHz, CDCl_3) δ 152.1, 150.1, 134.6, 130.7, 129.9, 129.6, 129.5, 128.6, 123.2, 118.5, 115.3, 45.9, 45.4, 24.8; ESI MS ($\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_2\text{S}_2$): m/z 309.1 $[\text{M} - \text{H}]^-$.

NMR Experiment. In a 3 mm NMR tube, 100 μL of DCYA (100 mM in CDCl_3) was mixed with 100 μL of electrophile (100 mM in CDCl_3) to give a final concentration of 50 mM for both DCYA and the electrophilic compound. Control ^1H NMR spectra were recorded every 5 min. After 90 min, a catalytic amount of the base DBN was added (10 μL of 100 mM DBN in CDCl_3 , 0.1 equiv), and a series of spectra were recorded at 5 min intervals for a minimum of 90 min. All spectra were recorded at constant temperature. All experiments were conducted using the manufacturer's standard presaturation pulse programs, and experimental conditions are discussed in the NMR Spectroscopy section. The progress of the reaction was followed by the depletion of the (olefinic/vinyl proton or other significant peaks of interest) signal of the test sensitizer (electrophile). The spectra were phased, referenced, and integrated using MNova, Agilent VJNMR, and Agilent CRAFT data elaboration software. Structural characterization analysis of the resulting adducts was confirmed by conducting additional 2D NMR experiments when necessary.

RESULTS AND DISCUSSION

To mimic skin chemistry, the majority of the state of the art in *chemico* methods are based on the depletion of the nucleophile of interest. However, these approaches are known for over or underestimation of potential skin sensitizers due to unwanted side effects such as drowning out and autoxidation of thiol nucleophiles.^{22,23,26,29} To overcome such difficulties, herein a method based on reactivity and classification of potential sensitizer by depending on depletion of electrophile signal (doEs) rather than the conventional depletion of nucleophile was employed. The proposed method takes advantage of unambiguous nuclear magnetic resonance spectroscopy. Additionally, NMR spectroscopy could serve as an ideal tool for accurately estimating the reactivity of electrophilic (sensitizer) candidates, the rate of reaction, and the quantification of NMR signal of interest. To explore the possibility of such a tool and to determine the optimum experimental parameters, the moderate sensitizer, *L* carvone **2**, was selected as a model electrophile and DCYA **1** was chosen as the model nucleophile (Scheme 1). The nucleophile, DCYA, was selected due to the biological relevance of cysteine, its ready solubility in common organic solvents, and its minimal interference with signals from the majority of test electrophiles.

Two arrays of spectra were collected: one as a (control) and a second one (reaction) after addition of a catalytic amount of DBN. To mimic the haptentation process, the organic base, DBN, was selected for activation of thiols at a molecular level rather than simulating physiological conditions. Both solutions were spiked with a known concentration of dimethyl furan as an internal standard. The change in concentrations of both electrophile and adduct were then plotted as percentage against time (in minutes). The concentration of vinyl proton of carvone in the control remained unchanged (Figure 2A and inset) for 90 min. Upon addition of the base, an immediate depletion (Figure 2B and inset) of carvone concentration was observed and continued for 300 min.

From Figure 3, it is evident that the reaction reached equilibrium at around 90 min and the same time was used as the cutoff

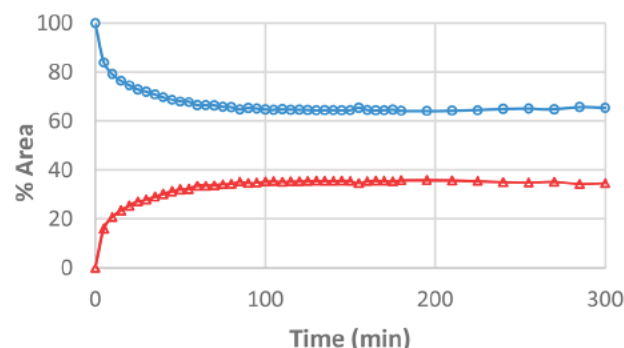


Figure 3. Percentage of the depletion of carvone's vinyl peak (blue circles) and the formation of the resulting adduct (red triangles) over a 300 min time frame.

time for all the test electrophiles except for the weak to nonsensitization candidates (monitored up to 24 h).

The initial results with *L* carvone led to further investigation of the effect of several critical parameters, such as type of thiol, base, solvent, temperature, and stoichiometry. The results of these studies are presented in Figure 4.

Type of Thiol. Although DCYA was the nucleophile of preference in the NMR study, to check comparative and compatibility issues, other thiol based nucleophiles were also investigated. The other candidates explored were an aromatic thiol (4 nitrobenzenethiol, NBT), the tripeptide glutathione (GSH), and *N* acetyl cysteamine (NAC). The choice of the solvent, DMSO d_6 , was determined by the poor solubility of some of the selected nucleophiles in many other deuterated solvents. Aqueous buffers would be a suitable choice, but because of many skin sensitizers have $\log P_{O/W} > 1$, a two solvent system would be required.⁴ The use of a two solvent system^{19,20,30} in NMR was not selected due to homogeneity, reactivity, overall kinetics, and other practical considerations. GSH is known to readily oxidized to form a homodimer,³¹ and dimerization is a significant limitation when attempting to quantify the depletion of GSH. In our NMR experiments, glutathione did not react with *L* carvone, and problems in quantification were observed due to low solubility and precipitation issues.

As anticipated, a slow reaction was observed with the aromatic thiol, NBT, under the tested conditions, with a conversion rate of <4% in 90 min. Aromatic thiols are known to be less reactive than aliphatic ones, and even if NBT is considered to be a surrogate for protein thiols,³¹ the long reaction times for estimating the reactive potential represent a major drawback for the NMR spectroscopic method. Reaction with NAC gave an initial 30% doEs, which eventually stabilized to around 37% doEs. The conversion rate was 10% higher compared to that for DCYA (Figure 4A), which slowly stabilized to around 25% doEs, and both are suitable test nucleophiles for the NMR method. However, DCYA was selected as the thiol of choice due to its distinctive NMR spectrum and minimal interference with the majority of electrophiles.

Basicity. The nucleophilic addition to *L* carvone is dependent on the activation of the sulfhydryl group to a thio anion (DCYA^-); thus, the reaction rate of carvone with DCYA^- following the addition of different base catalysts was examined. The calculated pK_a for DCYA is 10.1; thus, three different organic bases possessing comparable pK_a values and an aqueous inorganic buffer were chosen for further investigation. The role of basicity was evaluated using a catalytic amount (0.1 equiv for each 1.0 equiv of DCYA). The results shown in Figure 4B indicate that the tertiary

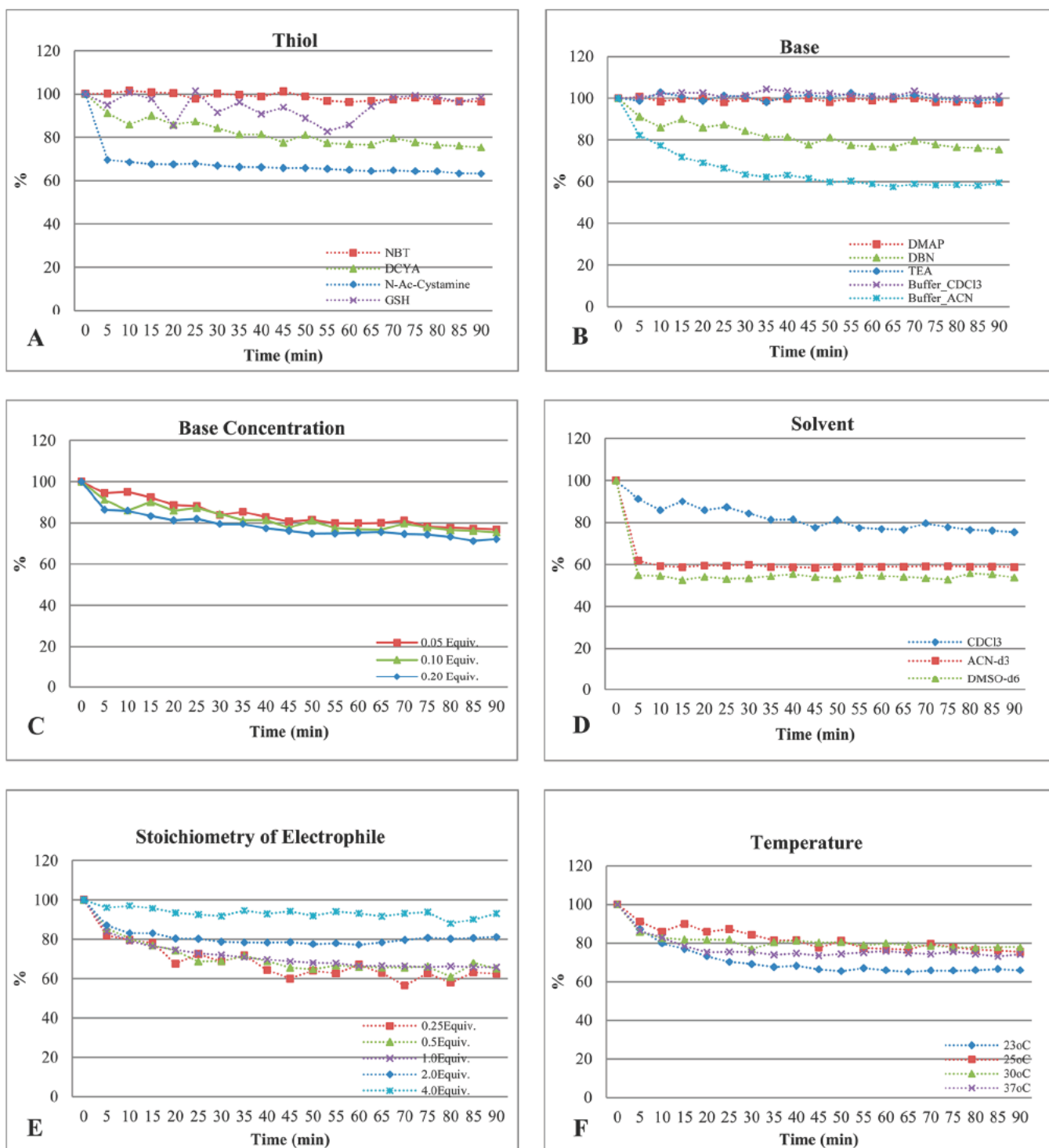


Figure 4. Effect of critical parameters on the outcome of adduct formation between DCYA and *L. carvone*. The percentages were calculated from the peak intensity of the vinylic proton of carvone (depletion of the peak at δ 6.8 with respect to the internal standard; dimethyl furan's peak is at δ 5.9 ppm). All results in the main text are expressed as the percentage of depletion of the electrophile signal (doEs).

amine, triethylamine (TEA, pK_a 10.8), and the aromatic amine, dimethylamino pyridine (DMAP, pK_a 9.2), did not show any catalytic effect under the experimental conditions, whereas the aqueous (5% v/v) pH 10 buffer/ACN d_3 was sufficient to catalyze the reaction in less than 5 min, with 42% depletion of the vinylic signal in 75 min. Similar results to the aqueous system buffer/ACN d_3 were observed using the organic base, DBN (pK_a 13.5), in chloroform, which gave 22% depletion. These results are in agreement with the reported base effect on thiol additions,³²

confirming the significance of basicity on the equilibrium between thiolate and thioether. The combination of DBN in CDCl₃ was thus considered to be the optimal conditions to avoid the possible interference of a two solvent system and the drowning out effects when using the NMR method.

Base Concentration. The effect of DBN concentration was evaluated on *L. carvone* and DCYA in chloroform *d* (Figure 4C). Variation of the concentration of DBN (0.05, 0.1, and 0.2 equiv with respect to 1.0 equiv of DCYA) showed depletions of 22, 25,

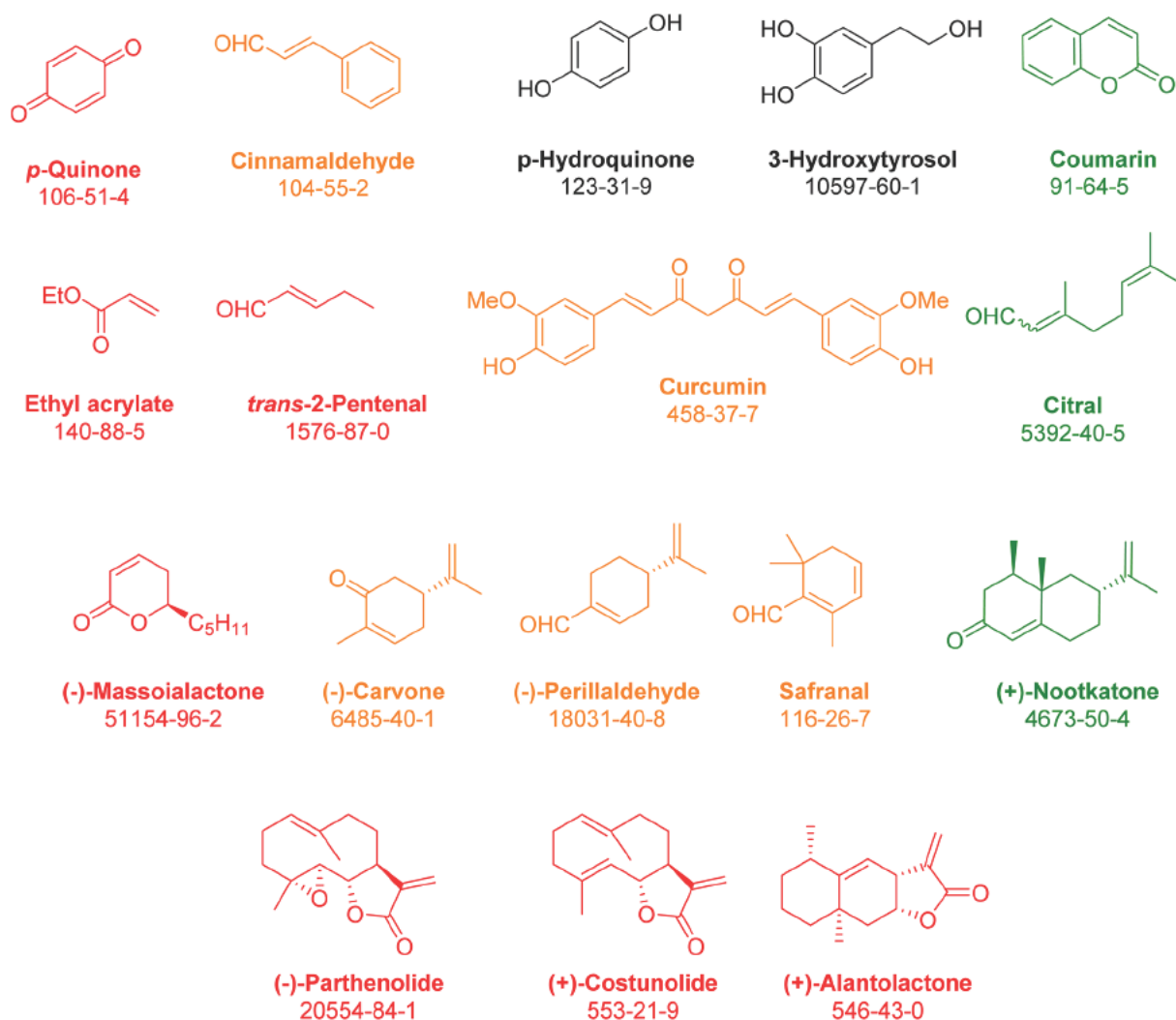


Figure 5. Tested sensitizers for reactivity and classification using NMR spectroscopy.

and 26%, respectively, with 0.1 equiv of DBN being the best condition in terms of reaction conversion in a short time frame. The optimum conditions were then chosen as 0.1 equiv of base for each 1 equiv of nucleophile, DCYA.

Solvent. The solvent effect was investigated using 0.1 equiv of DBN, 0.1 M DCYA, and 0.1 M *L* carvone in various solvent systems. The polar, aprotic solvents DMSO and acetonitrile as well as the nonpolar solvent chloroform were tested (Figure 4D). After 90 min, all three solvents gave a reasonable depletion, 46, 41, and 25%, respectively. All solvents were suitable in terms of *doEs*; however, because of its high solubility for electrophiles and minimal interference in ^1H NMR experiments, chloroform *d* was selected as the solvent of choice.

Stoichiometry and Temperature. Stoichiometries for *L* carvone/DCYA (1:4 to 4:1 ratios) were investigated by varying the concentration of carvone in the presence of 100 mM DCYA and 10 mM DBN (Figure 4E). In the presence of an excess of DCYA, the percentage depletion stabilized at around 37%. On the other hand, the excess of electrophile negatively affected thioether formation, which decreased to 19 and 7% with 2 and 4 equiv of carvone, respectively. The present work is mainly aimed at demonstrating the principle, so a 1:1 mol ratio was used; we envisage modifying the method to run under pseudo first order conditions (4–5 fold excess of DCYA) to reflect the function of reactivity and hence to be less influenced by equilibrium effects

and competing reactions. Variations in the temperature from 23 to 37 °C had only a limited effect on the depletion; thus, further experiments were carried out only at 23 °C (Figure 4F).

Screening of Potential Sensitizers. A number of potential skin sensitizers (Figure 5) were investigated for their depletion potential using the optimized NMR conditions. The compounds have been grouped according to their chemical structure, and the depletion results are shown in Figure 6.

Aromatic Compounds. Compounds containing α,β unsaturated ketones in extended conjugation with an aromatic moiety were herein grouped as aromatic compounds. The type and position of the extended conjugation may play a critical role in determining the electrophilic behavior of the candidate compounds. Many of these compounds, like the moderate sensitizer cinnamaldehyde,³⁹ could be formally classified in multiple mechanistic domains, and such ambiguity may be a critical limitation when using indirect assays or *in silico* prediction.⁴⁰ Moreover, compounds like cinnamaldehyde and *p* benzoquinone are also known for their oxidizing properties.²⁰ In the current NMR method, the reaction of DCYA with cinnamaldehyde gave 50% depletion after 30 min, whereas 95% depletion was observed for *p* benzoquinone, indicating the relevance of the NMR data with biological classification of these two electrophiles as moderate and strong skin sensitizers, respectively (Table 1).

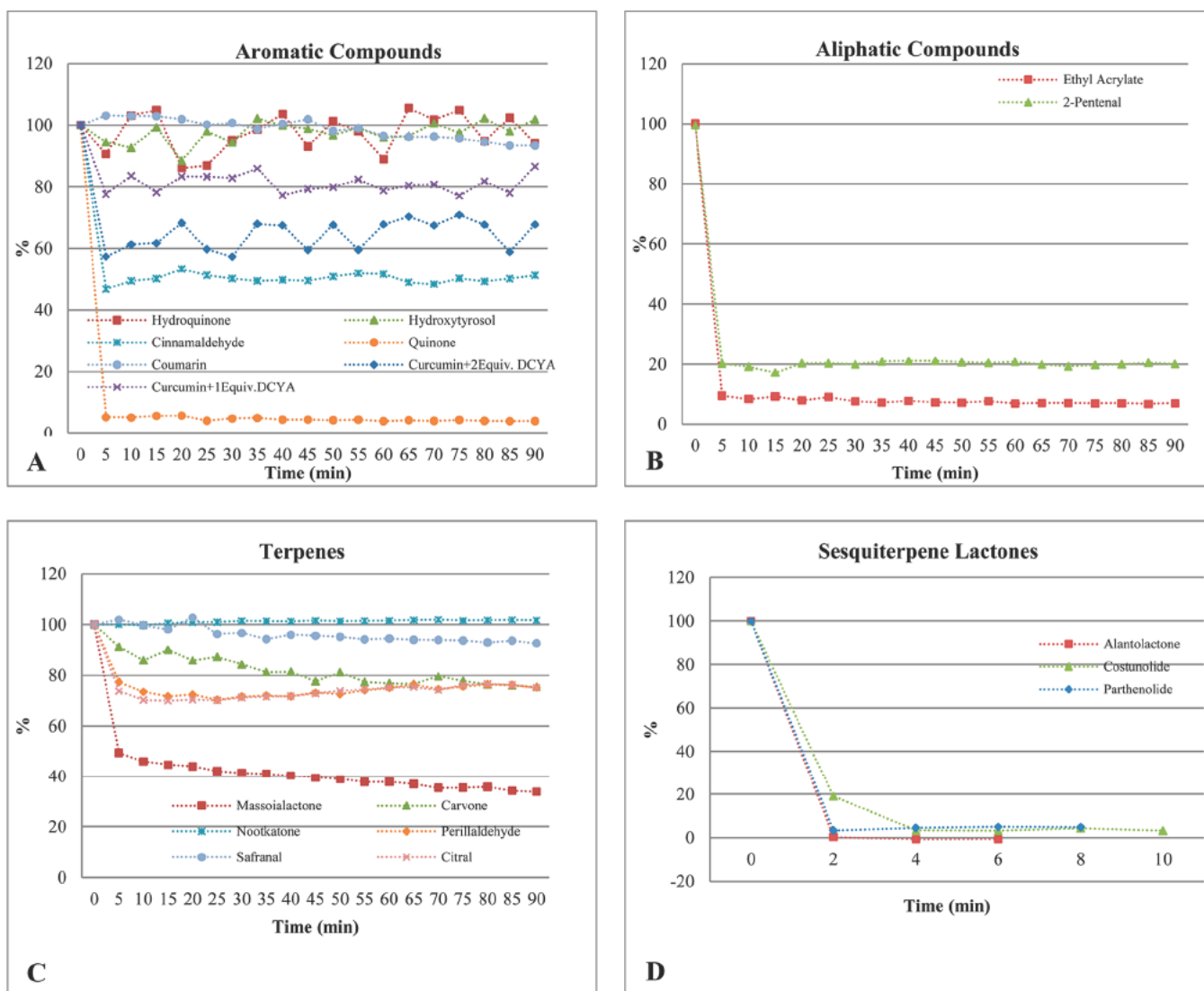


Figure 6. Depletion potential of tested compounds using the NMR method. The percentage of test chemical is expressed as the relative area of the vinylic or aromatic (for nonvinylic) proton plotted against time.

The scope of the NMR method for electrophiles with more than one reactive site was investigated. Interestingly, only weak to moderate reaction was observed for the phenolic natural product, curcumin, depending upon the number of equivalents of DCYA used. Due to the low solubility of curcumin in chloroform, DMSO d_6 was utilized as solvent, and 32% depletion was observed with 2 equiv of DCYA, whereas only 13% depletion was observed for 1 equiv of DCYA. The bis keto enone moiety can be formally classified as a double Michael acceptor site, which would make this molecule a potentially strong sensitizer. On the other hand, the structure of curcumin is known to exist in a keto enolic form rather than as a diketone.⁴¹ The resonance effect could have a negative impact on the electrophilicity of the double bond by interfering with its conjugation. Curcumin is also known for its antioxidant properties, where the central methylene group could act as a hydrogen donor through radical mechanisms.⁴² Altogether, these features make curcumin a complex, unpredictable skin sensitizer, where LC MS methods could actually fail to classify it. This result was somewhat unexpected considering that curcumin possesses multiple reactive sites, but it also explains the low incidence of skin sensitization reported for topical application of curcumin, considering its wide usage in traditional Eastern medicine.

Coumarin, hydroquinone, and hydroxytyrosol were found to be unreactive with DCYA. Coumarin is a known photosensitizer and has been earlier classified as skin sensitizer, but Vocanson et al.⁴³ demonstrated that this natural benzopyrone was not responsible for skin sensitization. Hydroxytyrosol and hydroquinone do not possess any classical electrophilic site(s) and are considered to be putative pre or pro haptens.^{33,34} The lack of reactivity measured by NMR is in agreement with other *in chemico* methods including DPRA results.³⁴

Aliphatic Compounds. One α,β unsaturated ester and one α,β unsaturated aldehyde were chosen for the study of aliphatic compounds. Ethyl acrylate is a volatile synthetic compound, which is known for its sensitization properties,^{44,45} and classified as a false negative in LLNA.³⁵ Ethyl acrylate gave 93% depletion, whereas the aliphatic aldehyde *t* 2 pentenal resulted 80% depletion. Skin sensitization data were not available on pentenal, but its homologue, hexenal, has been classified as a moderate sensitizer.⁴⁶

Natural Terpenes. The majority of natural sensitizers are volatile compounds belonging to the terpenoid class, and they are common constituents of various essential oils and fragrances. The two aldehydes, perillaldehyde and citral, both showed

Table 1. Comparison of Hapten Classification Obtained by the NMR Method with That from Published LLNA, DPRA, and KeratinoSens Data

test chemical	LLNA classification (EC ₃) ^a	NMR method (doEs) ^b	DPRA ^c	KeratinoSens EC ₃ [μ M]	ref and comments
3-hydroxy tyrosol	strong (0.6)	0	n.a.	n.a.	Pre/pro-hapten ³³
coumarin	non (NC)	7	1.0	479.96	ref 34
p-hydroquinone	strong (0.11)	6	83.3	51.29	Pre/pro-hapten ³⁴
safranal	moderate (7.5)	7	90.5 ²⁰	n.a.	ref 35
nootkatone	n.a.	0	n.a.	n.a.	
carvone	moderate (13 or 10.7)	25	26.3 ³⁶	258.71 ³⁶	ref 37
curcumin ^d	n.a.	13	n.a.	n.a.	
perillaldehyde	moderate (8.1)	25	31.9	61.85	ref 34
citral	moderate (9.2)	25	85.7	67.36	ref 34
curcumin ^e	n.a.	32	n.a.	n.a.	
cinnamaldehyde	moderate (3.0)	49	70.6	63.94	ref 34
massoia lactone	n.a.	66	n.a.	n.a.	
t-2-pentenal	n.a.	80	n.a.	n.a.	
ethyl acrylate	weak (28.0)	93	96.4	231.19	ref 34
parthenolide	n.a.	>95 [*]	n.a.	n.a.	positive in human patch tests ^f
p-benzoquinone	extreme (0.01)	96 ^h	99.0	32.77	ref 34
costunolide	n.a.	>97 ^g	n.a.	n.a.	positive in human patch tests ^f
alantolactone	n.a.	>99 ^g	n.a.	n.a.	positive in human patch tests ^f

^aEffective concentration is reported as percentage per microgram per cm². ^bDepleted percentage of test chemical at 90 min determined by NMR experiment. ^cPercentage of thiol peptide depleted (adopted from the literature). ^dOne equivalent of DCYA was used. ^eTwo equivalents of DCYA were used. ^fThese sesquiterpene lactones are reported to be positive in human patch tests.³⁸ ^gDue to their rapid reaction, spectra for these entries were collected for 10 min only. ^h5% of dimeric DCYA was also observed. NC, nonclassified; n.a., not available.

moderate/low electrophilic behavior (25% doEs), and both compounds were classified as moderate sensitizers according to LLNA and KeratinoSens results.^{34,46} The moderate sensitizer, safranal, was found to be less reactive and hence was classified as a nonsensitizer in our NMR method; however, a higher amount of depletion was observed for >180 min time frame. Along with known sensitizers, massoia lactone and nootkatone were also investigated. For the highly substituted electrophilic double bond in nootkatone, as anticipated, no depletion was observed even after 24h, whereas the fragrance component, massoia lactone, resulted in >66% doEs, indicating its sensitization potential.

Sesquiterpene lactones (SL) are among the most common contact allergens isolated from plants,⁴⁷ especially from the Asteraceae family, one of the largest flowering plant families in the world. The occurrence of skin sensitization to SL is so widespread that a sesquiterpene lactone mix (costunolide, dehydrocostuslactone, and alantolactone)⁴⁸ and a Compositae mix have been developed as screening tools for clinical patch tests.⁴⁹ Parthenolide and herbal extracts containing sesquiterpene lactones are known to cause severe allergic contact dermatitis.⁵⁰ Structurally, the epoxide and the α,β unsaturated lactone ring of parthenolide can compete as reactive sites for nucleophilic addition. The majority of the reported methods that rely on mass spectrometry would reveal only the presence of an adduct and the depletion of thiol without providing any structural information. When tested in the NMR method with 1 equiv of DCYA, all sesquiterpene lactones rapidly reacted irreversibly (<5 min) with a disappearance of the signals for the exocyclic double bond.

Moreover, NMR indicated the exclusive regioselective formation of the parthenolide–DCYA adduct due to reaction at the exomethylene γ lactone site over the epoxide position. The specificity of the thiol attack was previously reported by using cysteamine as a nucleophilic trap for irreversible Michael acceptors.⁵¹ Neither dimerization of the thiol to disulfide nor lactone thiolysis was observed in all tested lactones, indicating

the selectivity for Michael addition over other competitive reactions with candidates containing multiple electrophilic sites.

Overall, on the basis of the NMR method, all test chemicals were grouped based on the depletion (at t_{30}) of the electrophile signal and were categorized as non, weak, moderate, or strong entities. From Table 2, the NMR based classification appears to be reasonably reliable in delineating the categorization of potential sensitizers as having non, weak, moderate, or strong reactivity. However, the robustness, effectiveness, and limitations of this approach need to be tested further by applying it to a large set of potential sensitizers. In order to obtain a reliable reactivity index for potency estimation, the NMR methodology could be very easily adapted to evaluate kinetics and estimate the rate constant for each test substance.

CONCLUSIONS

In summary, a novel, biologically significant model nucleophile (DCYA) is proposed for use as a versatile trapping agent to identify and quantify the electrophilic potential of skin sensitizers. Several known skin sensitizers were evaluated and classified using a nuclear magnetic resonance spectroscopic method as an alternative *in chemico* method. The major advantage of the NMR method is the quantitation of the actual adduct. The degree of the reaction is provided here as a direct measurement of electrophile depletion, in contrast to other *in chemico* assays, e.g., ADRA and DPRA, where reactivity is inferred by quantifying the depletion of the test nucleophile. A kinetics version of the current NMR method could be extended to obtain a reliable reactivity index for potency estimation, and the accessibility of such resulting rate constants may be useful for the quantitative prediction of potency by QMM or read across methods. Because of the direct nature of doEs measurements, overestimation due to dimerization of the nucleophile and/or generation of insoluble adducts in categorization is minimal compared to that with existing methods. Moreover, the developed NMR method should serve as a qualitative and quantitative tool to understand the site of reaction

Table 2. NMR Classification of Potential Sensitizers Based on Depletion of the Electrophile Signal at 30 min

	Test chemical	doEs at t ₃₀	Classification
1	Hydroxytyrosol	6	Non ^s
2	Coumarin	0	Non
3	Hydroquinone	5	Non ^s
4	Safranal	3	Non
5	Nootkatone	0	Non
6	Carvone	16	Weak
7a	Curcumin	17 ^a	Weak
8	Perillaldehyde	28	Weak-moderate
9	Citral	29	Weak-moderate
7b	Curcumin	43 ^a	Moderate
10	Cinnamaldehyde	50	Moderate
11	Massoialactone	59	Moderate-strong
12	<i>trans</i> -2-Pentenal	80	Strong
13	Ethyl acrylate	92	Strong
14	Parthenolide	95	Strong
15	<i>p</i> -Benzoquinone	95	Strong
16	Costunolide	97	Strong
17	Alantolactone	99	Strong

[#]Only one equivalent of DCYA was used. ^{*}Two equivalents of DCYA were utilized. ^aPro electrophile sensitizers are likely to be falsely predicted as nonsensitizers.

and structural information associated with skin sensitizers. This is particularly valuable and advantageous over currently endorsed methods, which merely provide a quantification of the reaction but lack any structural information.^{52,53} Some of the compounds successfully tested here can be classified into more than one mechanistic domain and may be challenging to identify and classify using *in silico* or other non animal methods. As a proof of concept, the NMR method has been applied to compounds that are considered to be Michael acceptors. Other mechanistic domains, such as S_N2, Schiff base initiators and acylating agents, still need to be investigated to assess the broader scope of the NMR method.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.chemrestox.5b00098.

Spectra along with parameters, data, and data elaboration methods for all tested compounds (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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■ ABBREVIATIONS

ACD, allergic contact dermatitis; ADRA, amino acid derivative reactivity assay; DBN, diazabicyclo[4.3.0]non 5 ene; DCYA, dansyl cysteamine; DCYA E, dansyl cysteamine electrophile adduct; doEs, depletion of electrophile signal; DPRA, direct peptide reactivity assay; E, electrophile; ECVAM, European Centre for the Validation of Alternative Methods; LLNA, local lymph node assay; NMR, nuclear magnetic resonance; OECD, Organization for Economic Co operation and Development

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IKHLAS A. KHAN Ph.D.

FDA Center of Excellence

National Center for Natural Products Research, Division of
Pharmacognosy and Research Institute of Pharmaceutical
Sciences, School of Pharmacy,
The University of Mississippi

National Center for Natural Products Research

History

- Research Institute of Pharmaceutical Sciences, est. 1964
- NCNPR proposal developed in mid 1980s
- USDA-ARS initiated site visit in 1988
- Facility planning funds appropriated, 1989
- Groundbreaking - October 20, 1990
- Construction 1990 - 2001
- Launch/partial occupancy - July 1995
- USDA-ARS Natural Product Utilization Research Unit (NPURU) located 1996
- Facility Dedication 1998
 - “Thad Cochran Research Center”

National Center for Natural Products Research

- People -
 - ~24 Ph.D. research faculty and scientists
 - > 45 technical, administrative staff, postdocs & visiting scientists
 - >25 USDA research scientists and technical staff
 - Graduate & undergraduate students
 - >25 faculty of the academic departments of the School of Pharmacy

National Center for Natural Products Research

Natural Product Discovery and Development

- ***Drug Discovery and Development*** - To discover single entity and multicomponent bioactive natural products that may serve as leads for the development of new pharmaceuticals. Emphasis is on agents that control certain infectious diseases, cancer, inflammatory or immune disorders.
- ***Agrochemical Discovery and Development*** - To identify lead compounds for the development of environmentally benign and toxicologically safe pest management agents. Emphasis is on agents useful in the control of pests affecting small niche crops.

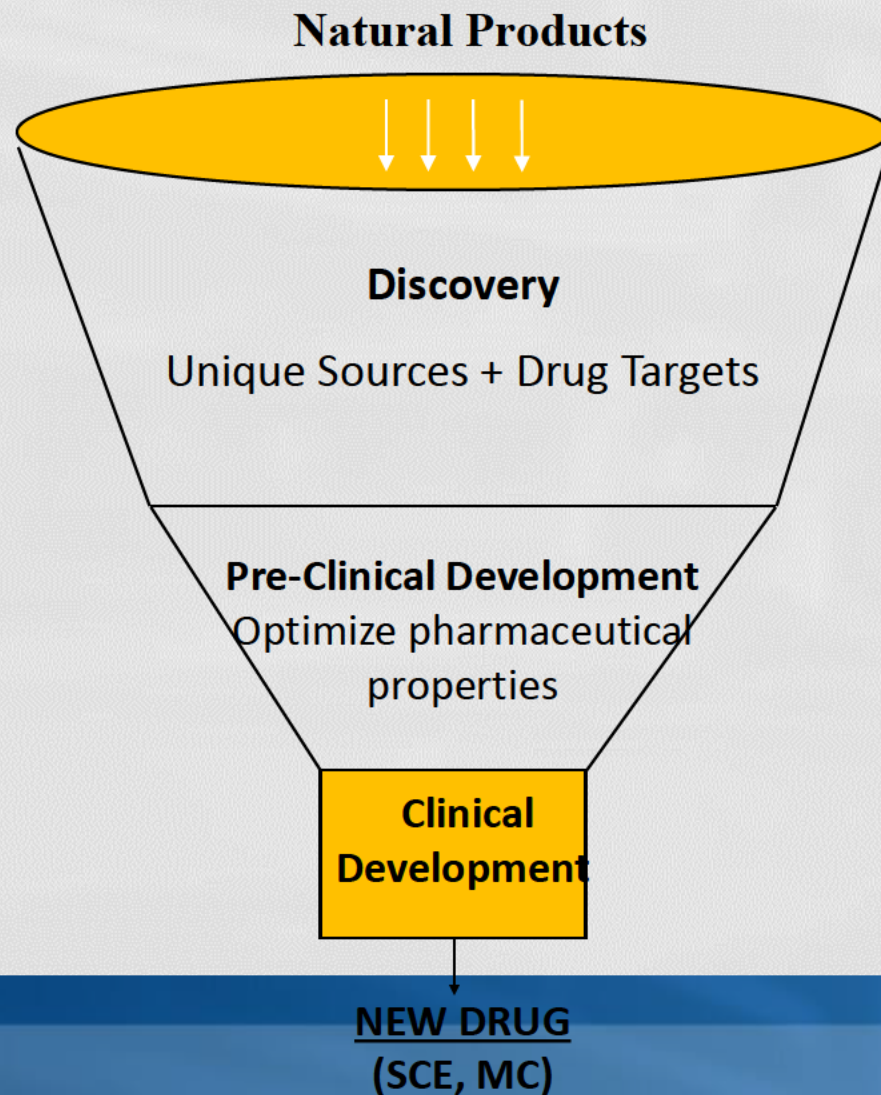
Medicinal Plant Research

- ***Medicinal Plants as Alternative Crops*** - To examine the biology, chemistry, and agronomics of medicinal plants so they may be developed as alternative crops for US farmers.
- ***Botanicals and Human Health*** - To identify botanicals with potential to improve human health and to enable safe, effective, and proper use of high quality botanical products by informed professionals and consumers.



National Center for Natural Products Research

Drug Discovery and Development Research



Natural Product Discovery and Development Research Program

Natural Product Repository

- More than 14,000 samples of extracts, derived fractions, and pure compounds
- ~6000 samples in archived 96-well microplates
- Taxonomic diversity - less than 10% of the specimens are derived from any one family
- Ongoing collection efforts to expand by 1000 - 2000 samples per year
- Development, implementation, and validation of rational collection strategies
- Geographic representation includes North America, South America, Central America, Africa, Papua New Guinea, India other sites
- Collaborations with other institutions provide access to ~ 20,000 additional samples (NCI, University of Auckland) for screening in the Center's bioassays (for both drug and agchem discovery)
- Samples are available to collaborators on an exclusive or non-exclusive basis for specific therapeutic areas

Medicinal Plant Research

- BOTANY
- HORTICULTURE
- AGRONOMICS
- PLANT GENOMICS
- PLANT TISSUE CULTURE
- NATURAL PROD. CHEMISTRY
- CHEMOTAXONOMY

- ETHNOMEDICINE/BOTANICALS
- ANALYTICAL CHEMISTRY
- REFERENCE STANDARDS
- BIOLOGICAL ACTIVITY
- INFORMATICS
- TOXICITY
- ADME



NEW or IMPROVED CROPS
NEW COMPOUND SOURCES



SAFER, MORE EFFECTIVE DIETARY SUPPLEMENTS



THE UNIVERSITY OF
MISSISSIPPI
National Center for
Natural Products Research

Research Interests between Office of Cosmetics and Colors/ CFSAN & NCNPR/ U of Mississippi



Specific Aims

- Assist in the identification and development of a list of BDS and botanical ingredients, based on **safety concerns**, trends, and knowledge of **botanicals being marketed** in the U.S. to prioritize for further research.
- Acquire, validate, and characterize **authenticated reference materials**, including raw and processed plant materials and purified natural products of relevance to the FDA, for evaluation of their safety.
- **Exchange technical and scientific information**, analytical methods, and reference material with FDA scientists and other stakeholders.
- **Collaborate with FDA scientists** in research areas of mutual interest.
- Coordinate **scientific workshops and conferences** on BDS-related topics of public health relevance to address high priority science and research needs.

FDA's BDS Center of Excellence



FDA's BDS Center of Excellence

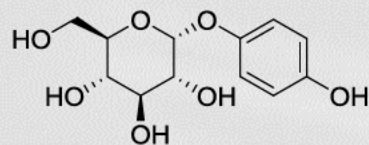


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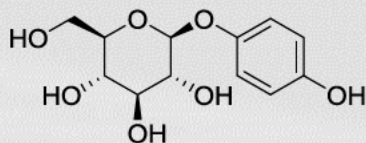
Tasks

- **Task #1:** Arbutin in skin whitening products
 - Natural
 - Other forms
 - Chemical/Enzymatic Stability
 - Marketed Products
- **Task #2:** Are there any potential chamomile side effects or risks?
 - Which chamomile?
 - Roman? or German? or Chinese
 - Skin sensitization
 - Alternative methods
 - KeratinoSens or DPRA
 - IP on UM-NCNPR methods
 - Tonghaosu from German
- **Task #3:** Tea tree oil- Adulteration, substitution and adverse effects
 - Malaleuca
 - Alternifolia? or Linariifolia or Viridiflora, or??
 - Potential sensitizers
 - Application of UM-HTS method
 - Stability and Storage of Terpenes

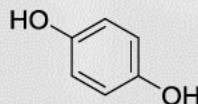
Arbutin in Cosmetics



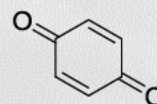
β -Arbutin



α -Arbutin



1,4-Hydroquinone



p-Quinone

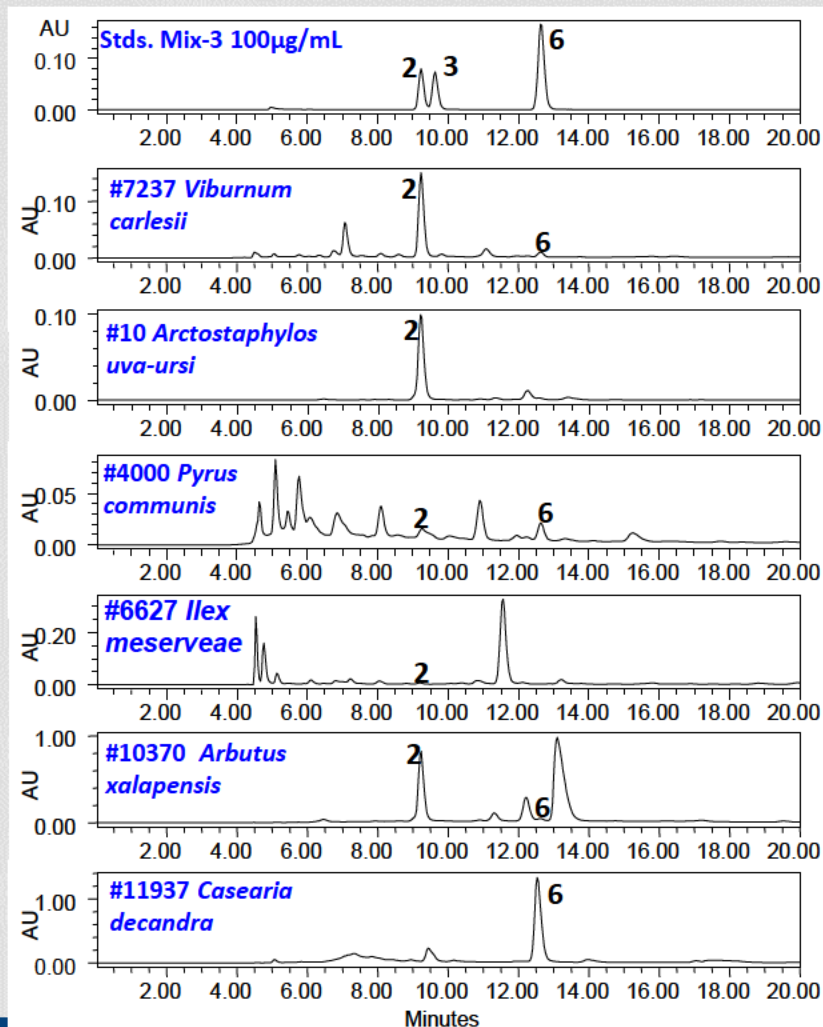


- Arbutin is the glycoside derivative of hydroquinone. The β -glycoside anomer is commonly found in species of several plant families in nature, whereas α -arbutin can be obtained by biotransformation or by chemical synthesis.
- Both β - and α -arbutin became popular skin whitening agents because of their ability to interfere with melanin synthesis.
- Upon on topical application, arbutins can be hydrolyzed to hydroquinone, which is known to cause ochronosis and leukomelanoderma .
- In order to assess the stability of α - and β -arbutin, both compounds have been investigated for their potential hydrolysis to release hydroquinone



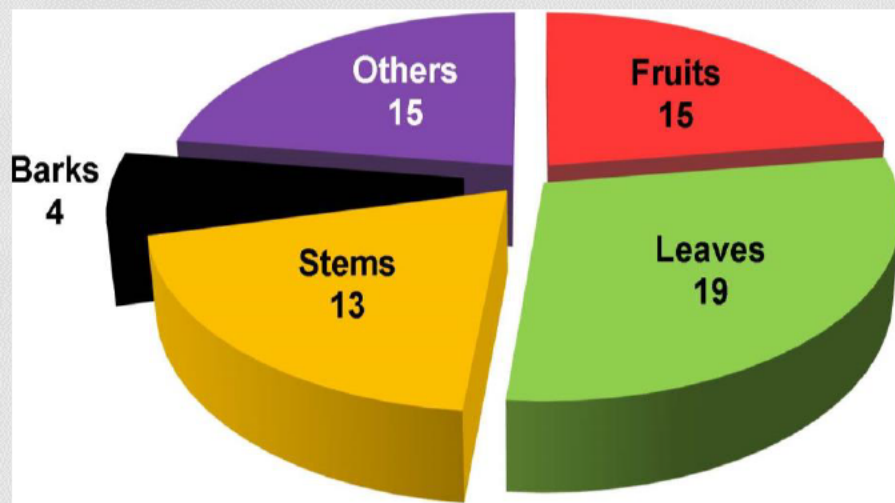
Arbutin from Plant

HPLC-UV chromatograms of β -arbutin (2), α -arbutin (3) and hydroquinone (6) from various species



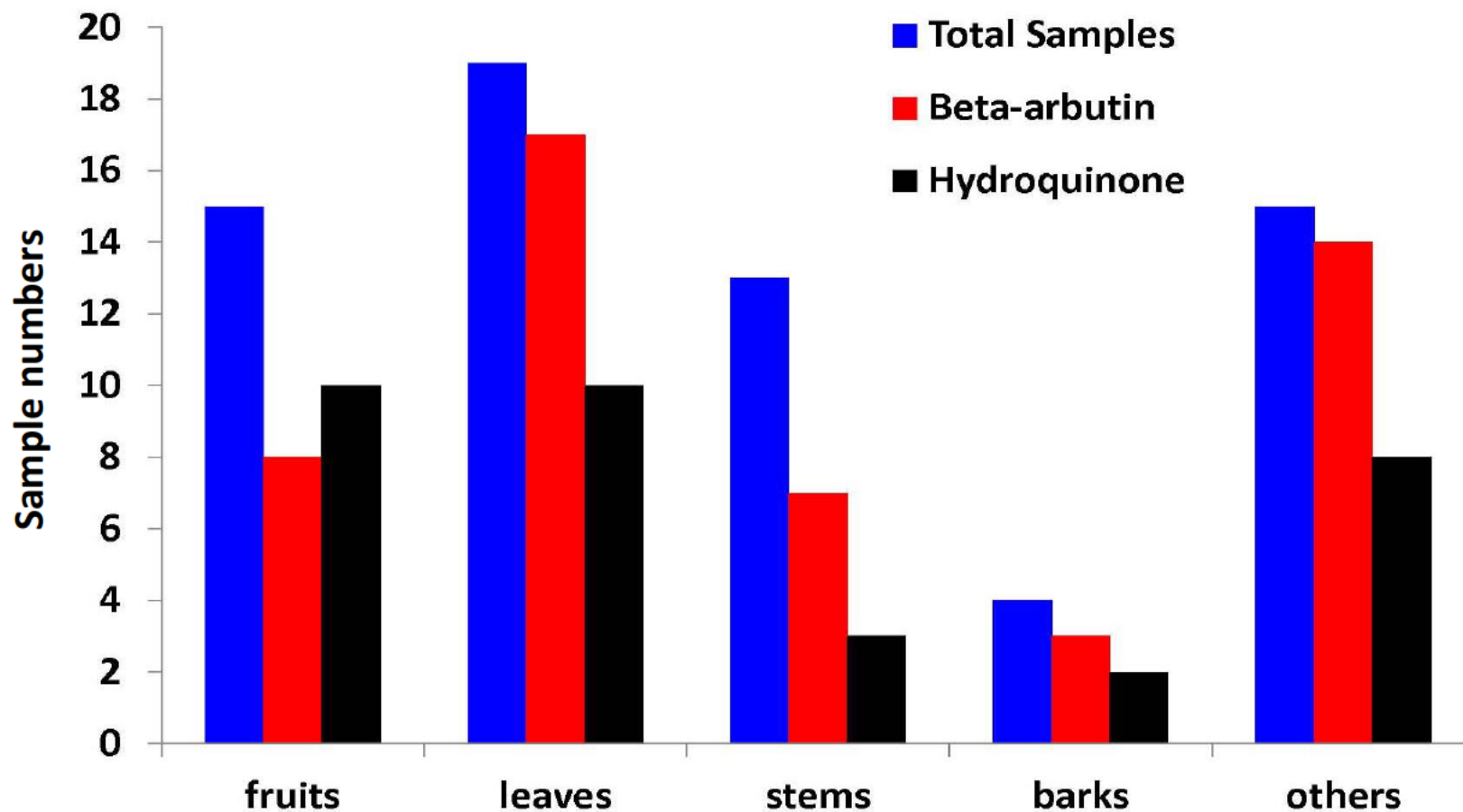
of samples analyzed for different plant parts

Total samples = 66

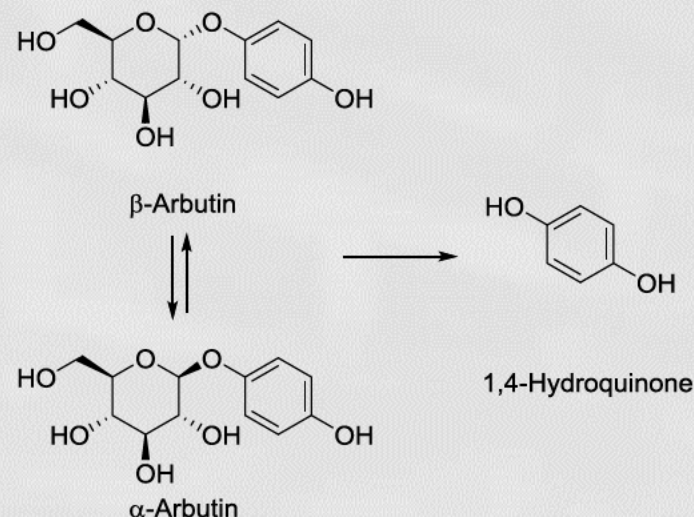


Arbutin from Plant

of samples analyzed for different plant parts and samples containing β -arbutin and hydroquinone

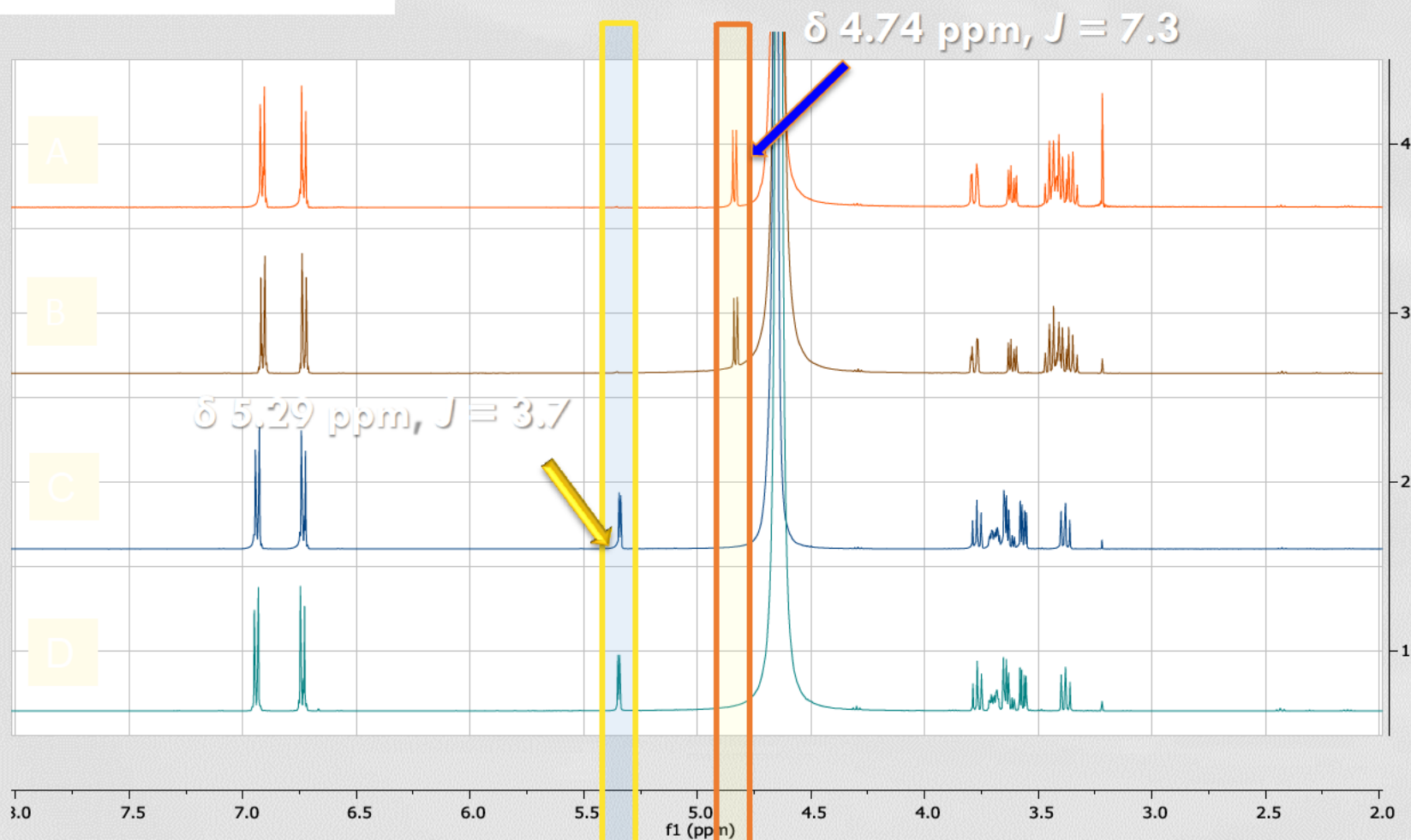


Arbutin in Cosmetics



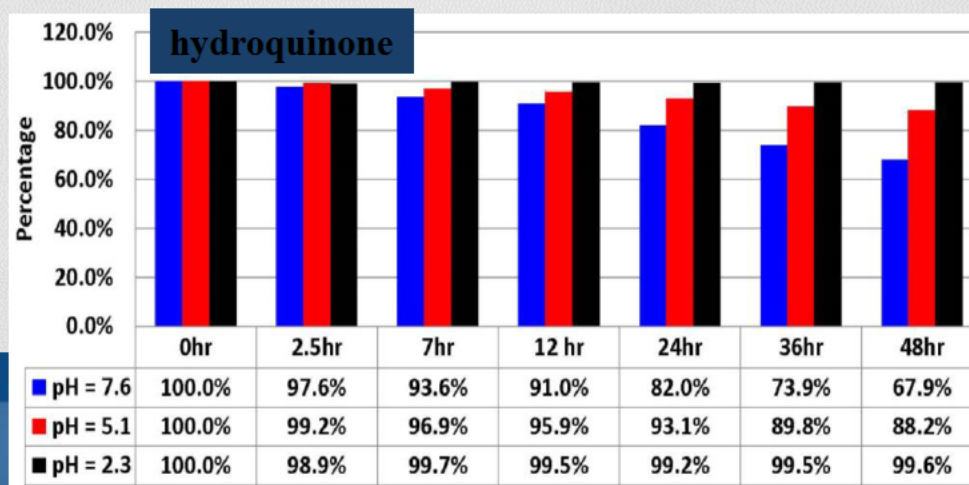
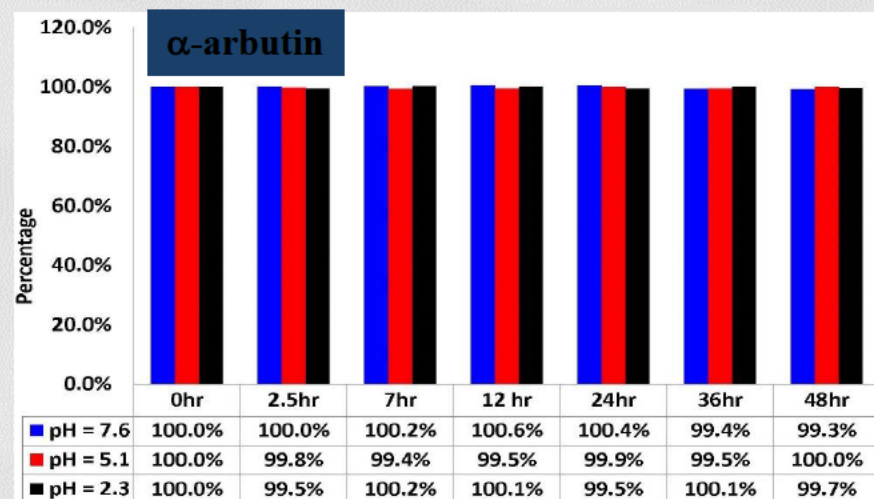
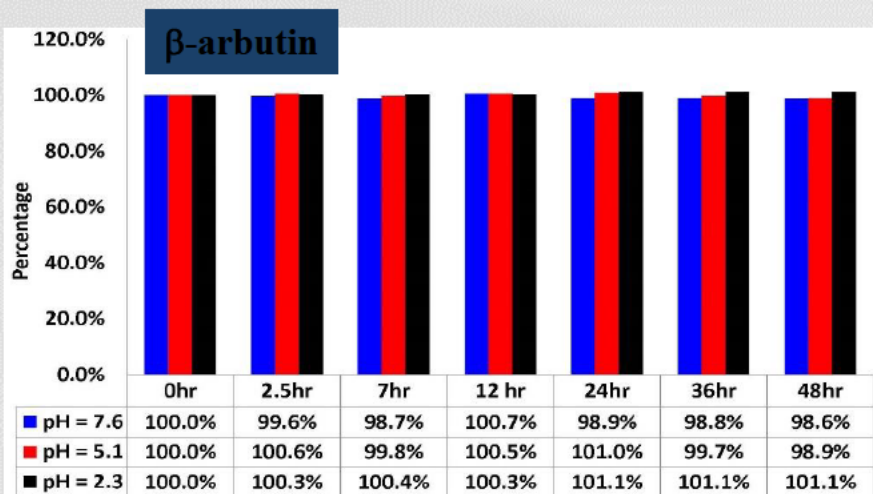
- Both α - and β -arbutin were found to be stable in methanol or water solutions during 6 months storage under artificial light
- No interconversion of α/β -arbutin, nor release of hydroquinone was found under tested conditions.
- Strong pH conditions are found to have deleterious effects on the stability of arbutins.
- Enzymatic stability studies were performed using fresh pear peels as a surrogate of a biological system. Around 50% loss in 4h, and > 90% in 24 h was observed for β -arbutin. Both α - and β -arbutins were found to be stable under deactivated enzymatic conditions

^1H NMR stability test



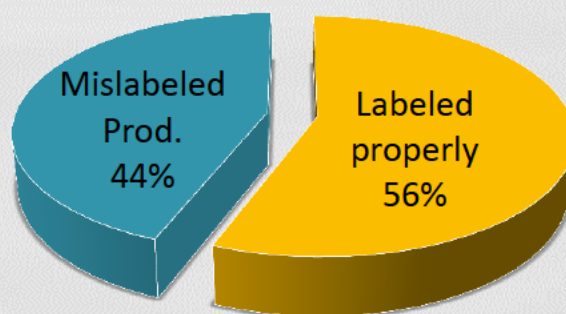
Stability of β - and α -arbutin in D_2O : (A) Standard β -arbutin; (B) sample A stored for 6 months in solution; (C) Standard α -arbutin; and (D) sample C stored for 6 months in solution.

Stability test of β -arbutin, α -arbutin and hydroquinone in 48 hr at different pH by HPLC-UV



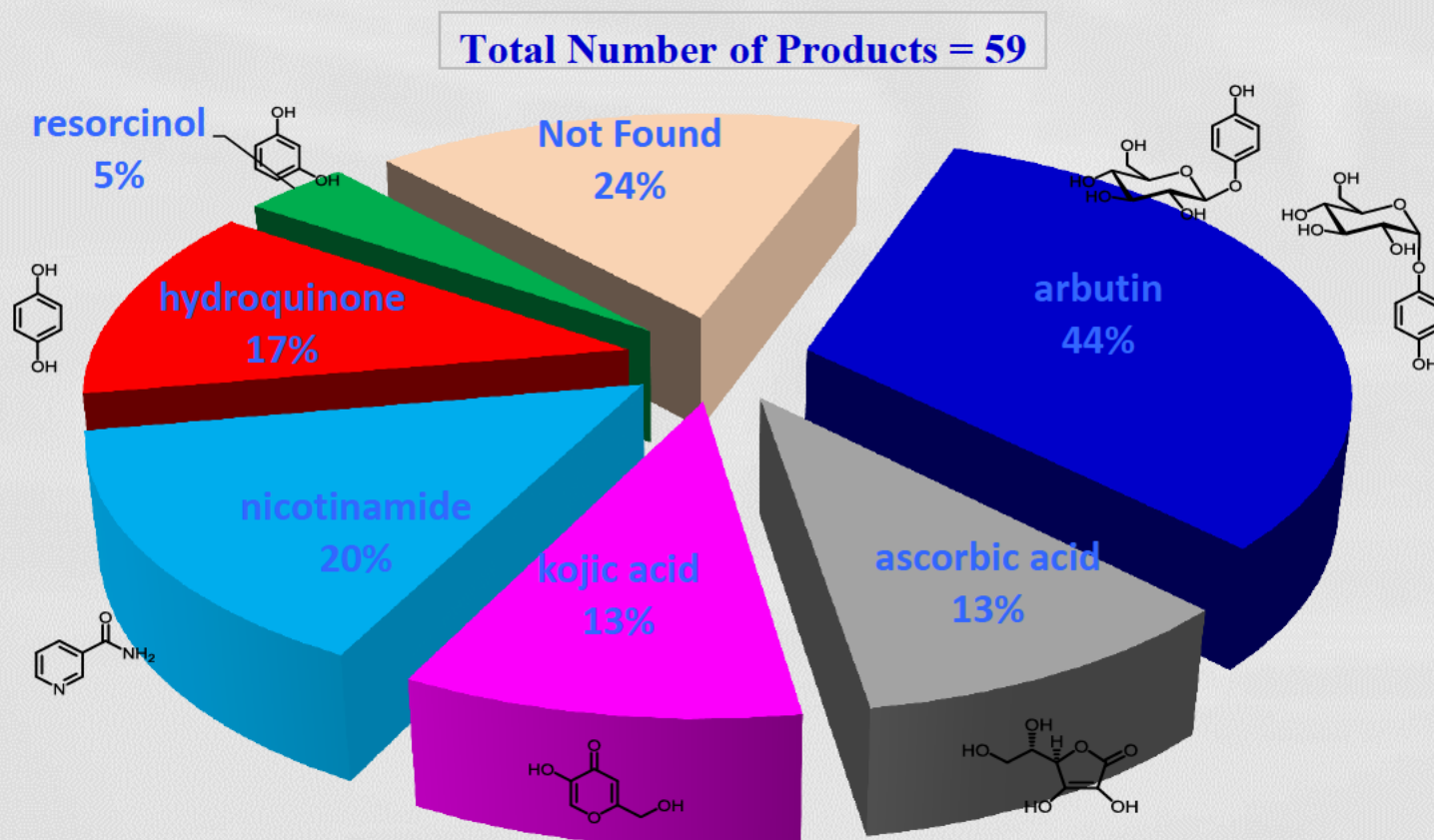
Skin Whitening Products

- The developed HPLC-UV method was applied to the quantitation of nine analytes in 59 skin whitening products including creams, lotions, sera, foams, gels, mask sheets, soap bars, tablets, and capsules.
- Arbutins (β - and/or α -), ascorbic acid, kojic acid, nicotinamide, hydroquinone, and resorcinol were identified in 27, 8, 8, 13, 10, and 3 products, respectively. No sample contained 4-methoxyphenol or 4-ethoxyphenol.
- From an overview of the 59 whitening products, more than 40% of the products were mislabeled.



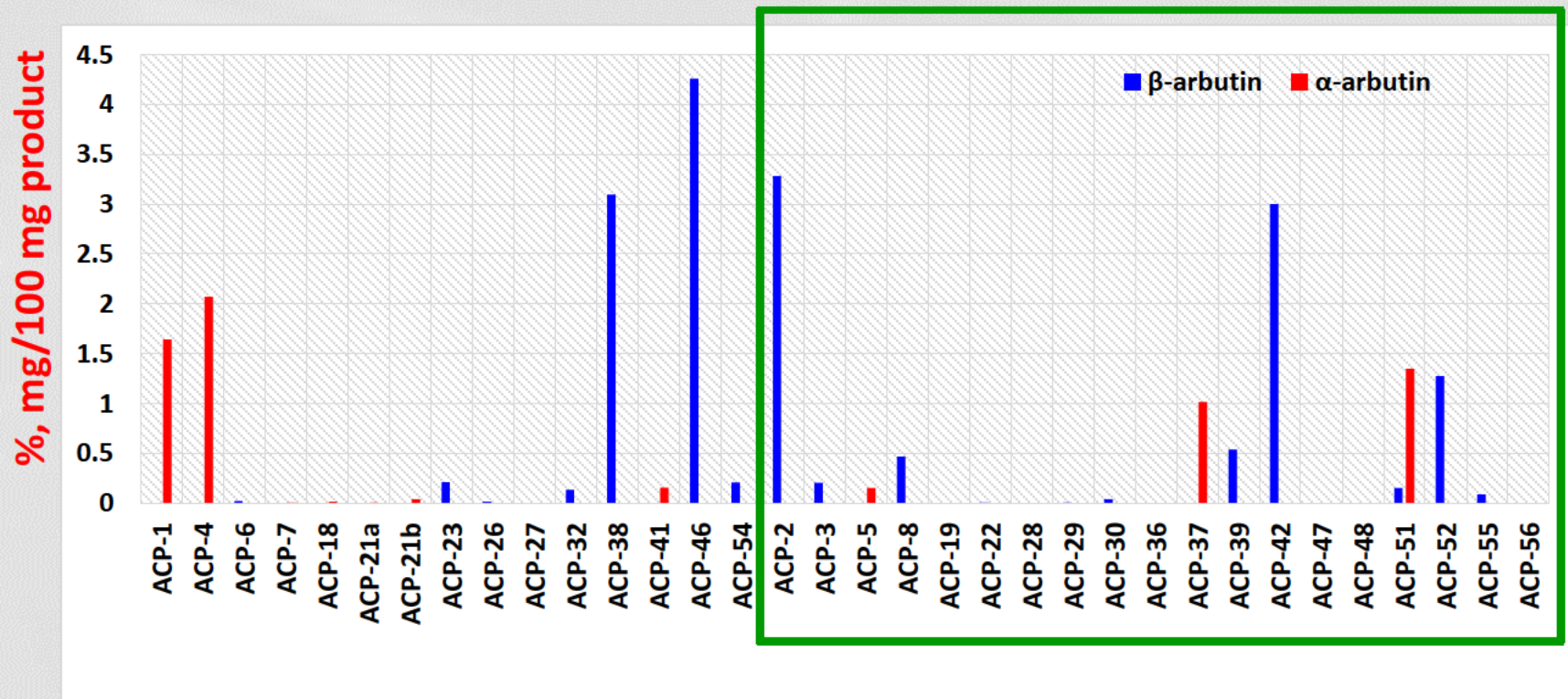
Skin Whitening Products

Total Distribution of Individual Analytes



Skin Whitening Products

Distribution of Individual arbutin isomers



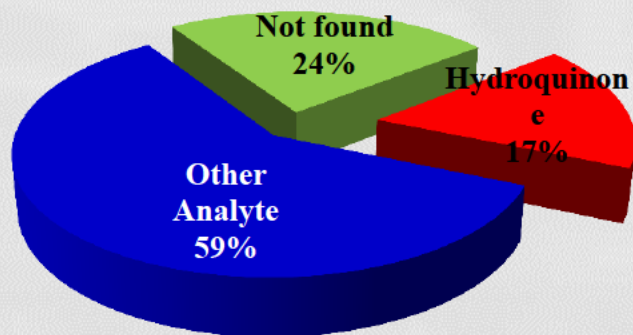
- Highlighted products, 19 out of 34 products, **showed labeling problems** in the following aspects: 1) missing the labeled ingredient; 2) detection of β-arbutin instead of labeled α-arbutin; 3) extra analyte found but not mentioned on the label.

Whitening Products: Safety Concern

Levels of Hydroquinone

Products Found Hydroquinone in Total Products

Total Number of Products = 59



- One product contained 3.1% hydroquinone which was 163% of the listed amount on the product label.
- Hydroquinone in the range of 1.4 – 1.9% was found in four products.
- Three products were found to contain hydroquinone that was not listed on label.

Chamomile

Classification of Chamomile Flowers, E. Oils and Comm. Products

- Two most popular and commonly used chamomiles
 - German chamomile (*Matricaria chamomilla*, synonym: *Matricaria recutita*)
 - Roman chamomile (*Chamaemelum nobile*, synonym: *Anthemis nobilis*)

- Methodologies Applied:

- GC-MS
- UPLC-QToF
- HPTLC



Matricaria chamomilla



Chamaemelum nobile

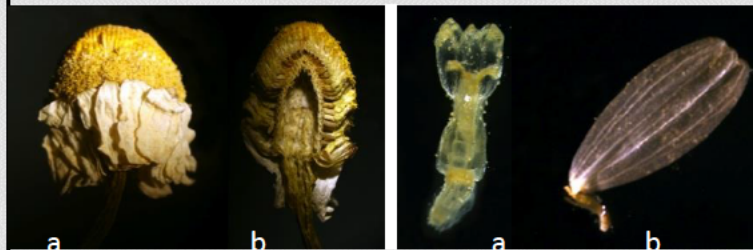
Macroscopy

German Chamomile (*Matricaria recutita*)



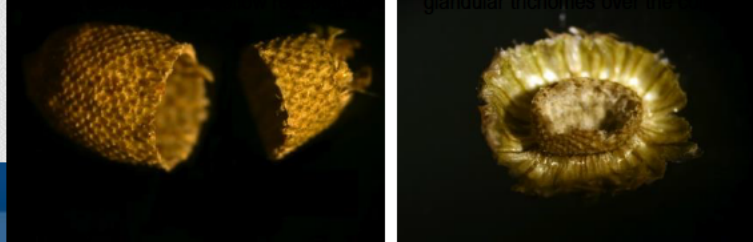
Habit

Raw drug (dried flower-heads)



Flower-head: entire (a) and vertically cut (b) to show the arrangement of florets as well as the hollow receptacle

Disc (a) and ray (b) florets in magnified view. Note: observe the glandular trichomes over the receptacle



Receptacle (florets removed): cut open to show the hollow center. Palea absent.

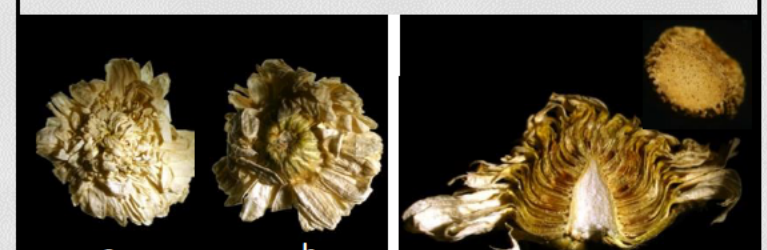
Involucre bracts and a portion of receptacle

Roman Chamomile (*Chamaemelum nobile*)



Habit

Raw drug (dried flower-heads)



Flower-head: views from above (a) and below (b)

Flower-head: vertically cut to show the solid receptacle, arrangement of florets (note all rayed) and palea

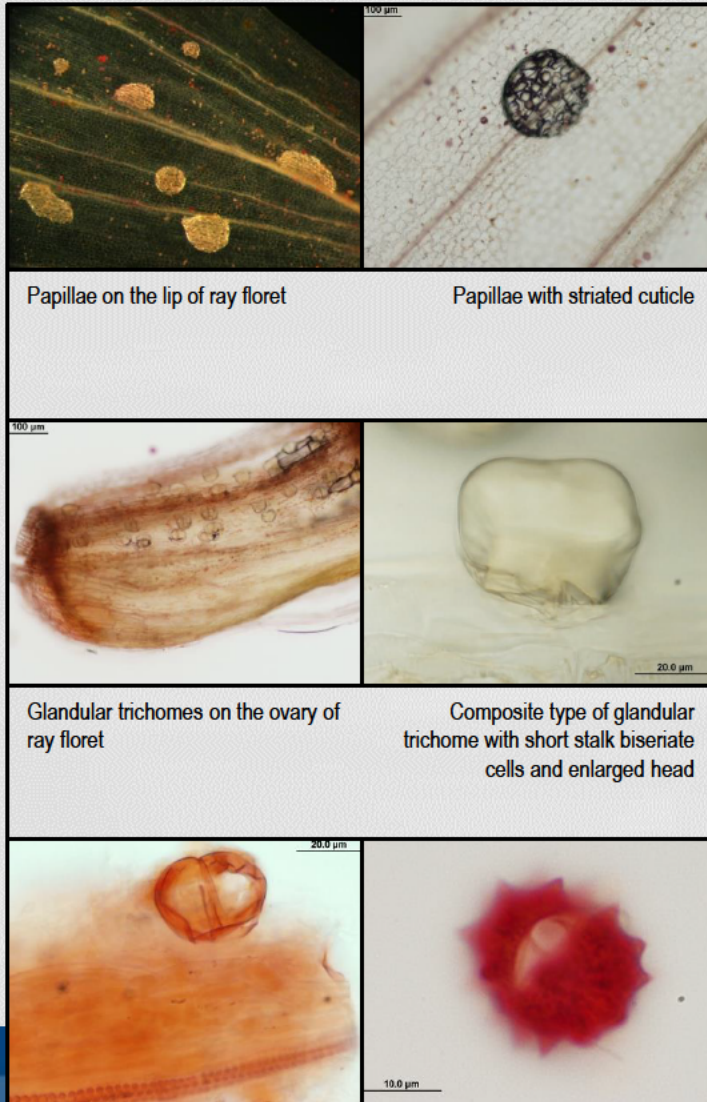


Ray florets from (a) outer and (b) inner whorls

Involucre bract (a) and palea (b)

Microscopy

German Chamomile (*Matricaria recutita*)



Papillae on the lip of ray floret

Papillae with striated cuticle

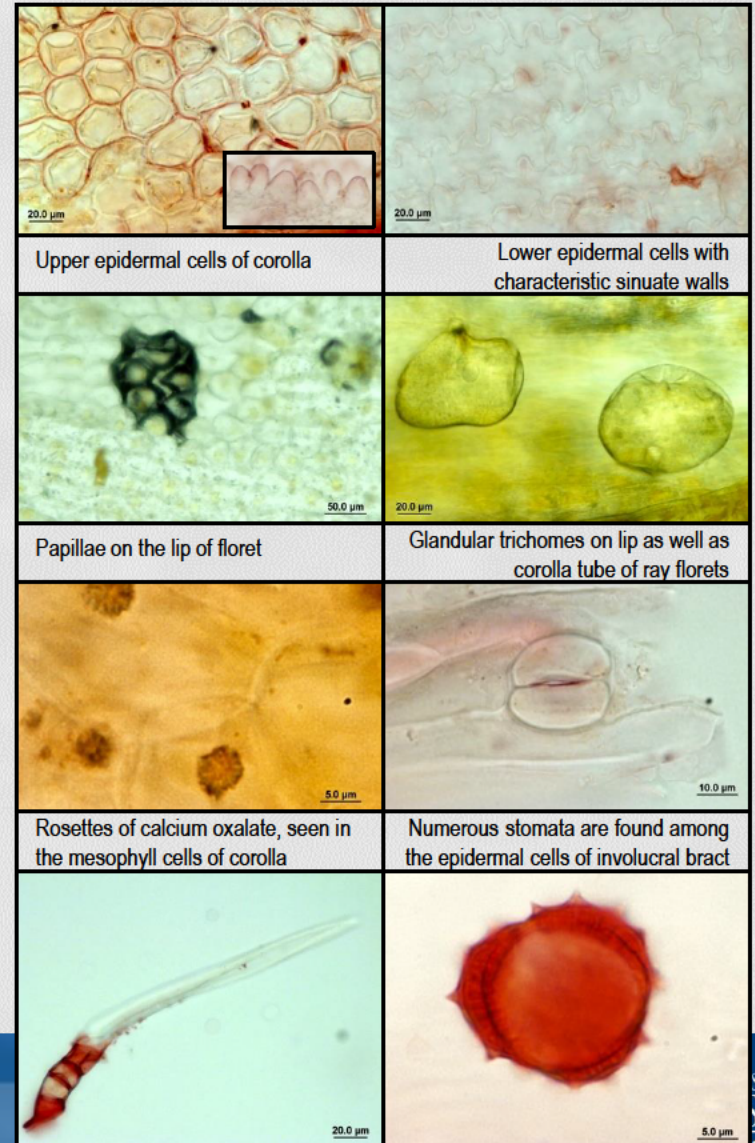
Glandular trichomes on the ovary of ray floret

Composite type of glandular trichome with short stalk biseriate cells and enlarged head

Glandular trichome with ruptured cuticle

Pollen grain showing one of the three germ pores. The exine is spinulose.

Roman Chamomile (*Chamaemelum nobile*)



Upper epidermal cells of corolla

Lower epidermal cells with characteristic sinuate walls

Papillae on the lip of floret

Glandular trichomes on lip as well as corolla tube of ray florets

Rosettes of calcium oxalate, seen in the mesophyll cells of corolla

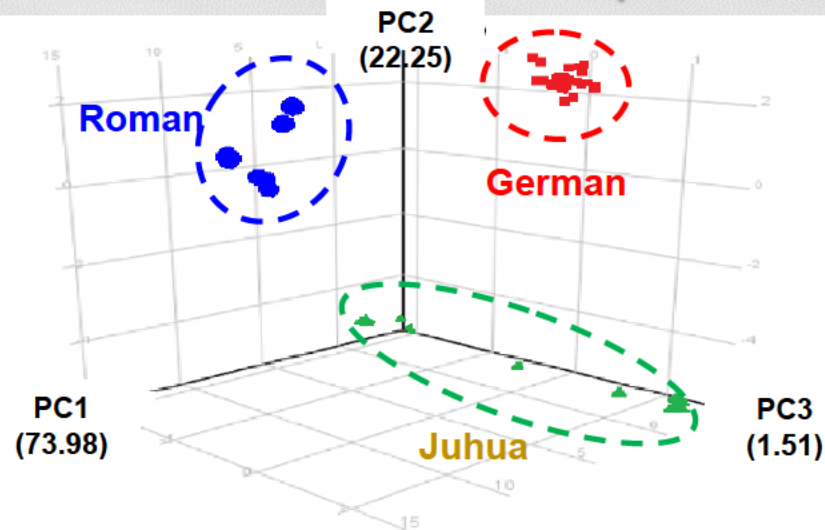
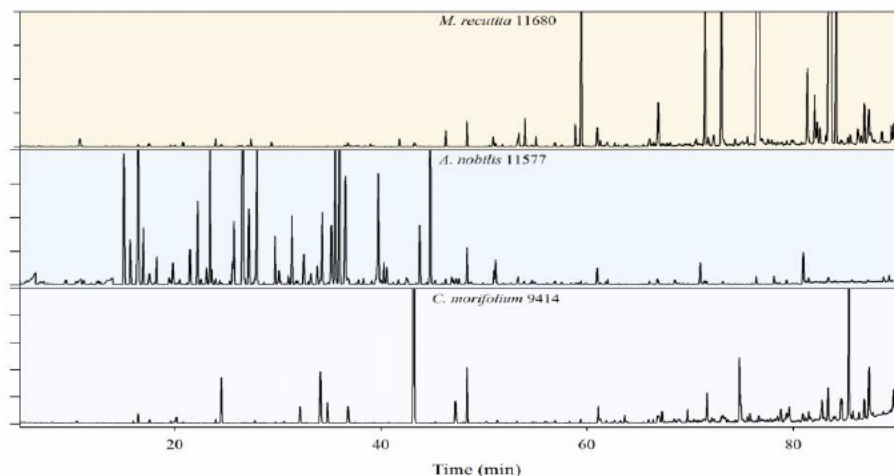
Numerous stomata are found among the epidermal cells of involucre bract

Non-glandular trichome

Pollen grain showing three germ pores, and spinulose exine

Chamomile

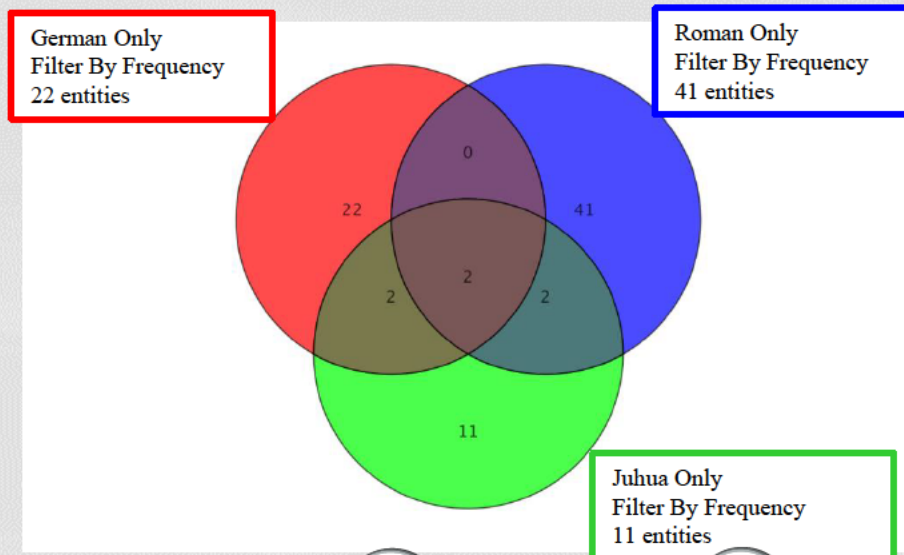
Classification of Chamomile Using Chemometrics and GC/MS



- A highly accurate statistical model has been developed to determine the exact type (**German, Roman and Juhua**) of chamomile used in commercial herbal products and dietary supplements.
- The model was developed from GC/MS data. Quality control of the samples was performed by Principal Component Analysis (PCA).
- A sample class prediction model based on Partial Least Squares Discriminant Analysis (PLS-DA) was constructed.

Chamomile

Classification of Chamomile Using Chemometrics and GC/MS



- 35 samples including chamomile flowers, extracts, teas, dietary supplements, and 11 chamomile essential oils were classified by the PLS-DA model
- 4 outliers have been identified. Age and storage conditions may have caused the issue
- The results suggested that **German chamomile** is the major type of chamomile used in the **U.S. market**.
- In contrast, all the chamomile samples purchased from China were identified as Juhua chamomile

Chemometrics can be used to analyze large, complex (3-D) data sets MUCH faster than manual analysis

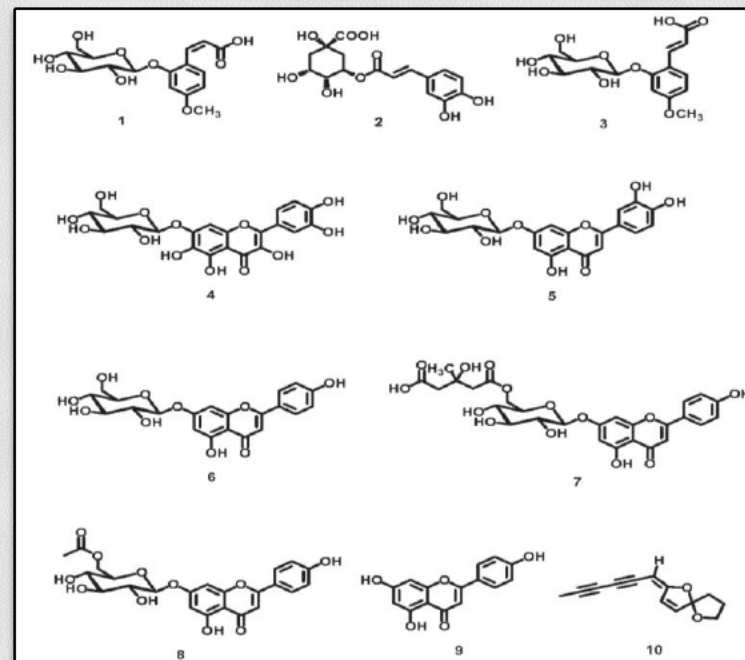
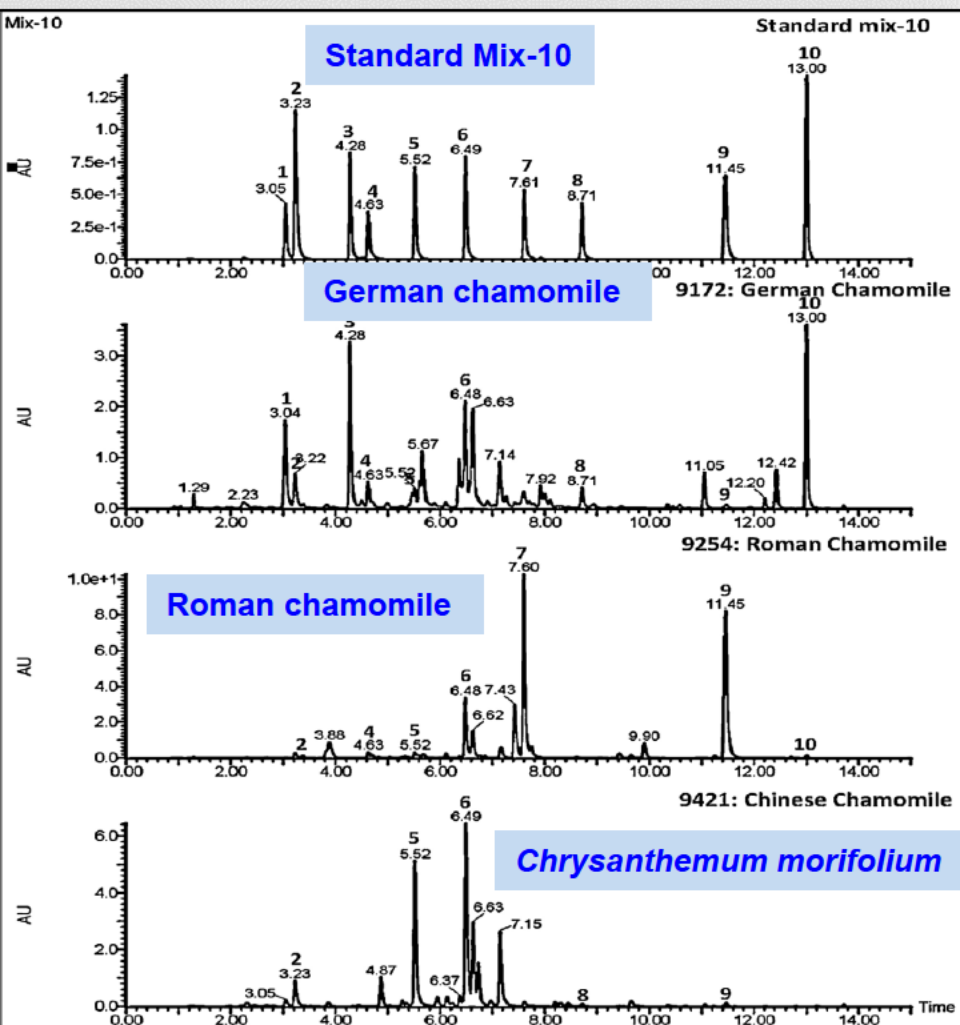
With AUTHENTICATED samples, an accurate sample class prediction model can be developed and verified

The SCP model can subsequently be used to analyze samples in an automated manner w/o reanalysis of the authenticated samples

Chemometric analysis can be used to identify potential markers for different type of samples

Chamomile

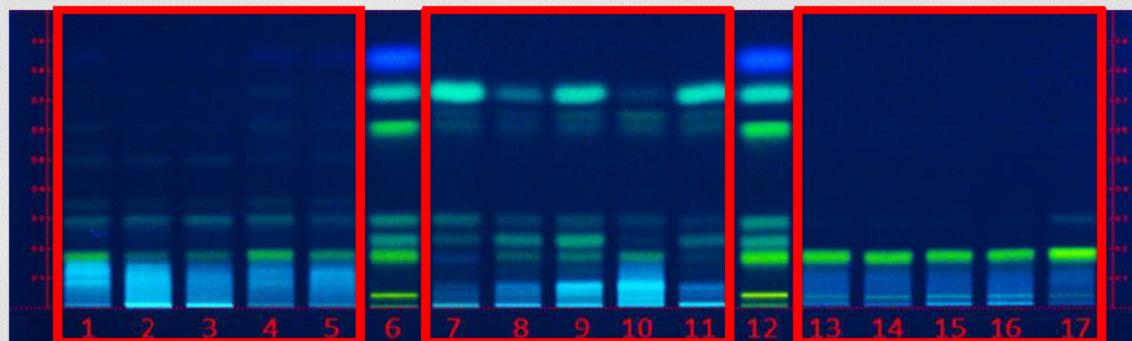
Classification of Chamomiles Using UHPLC-UV-MS



Standard compounds: cis-GMCA (1), chlorogenic acid (2), trans-GMCA (3), quercetagenin-7- β -D-glucopyranoside (4), luteolin-7- β -D-glucoside (5), apigenin-7- β -D-glucoside (6), chamaemeloside (7), apigenin 7- O -(6''- O -acetyl)- β -D-glucopyranoside (8), apigenin (9), tonghaosu (10).

Chamomile

Classification of Chamomiles Using HPTLC

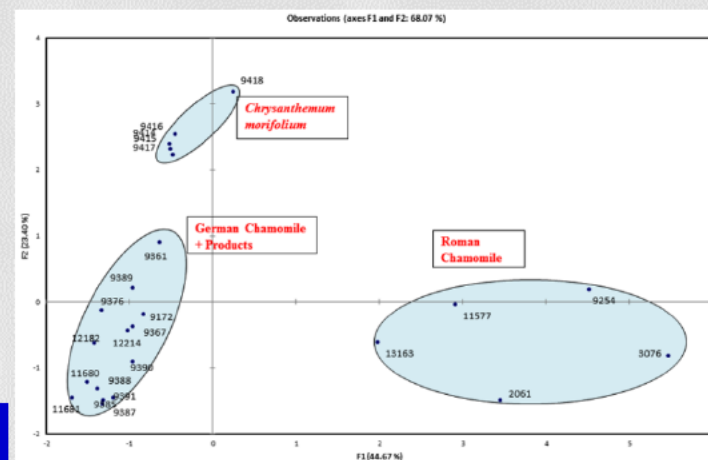


German
chamomile

Roman
chamomile

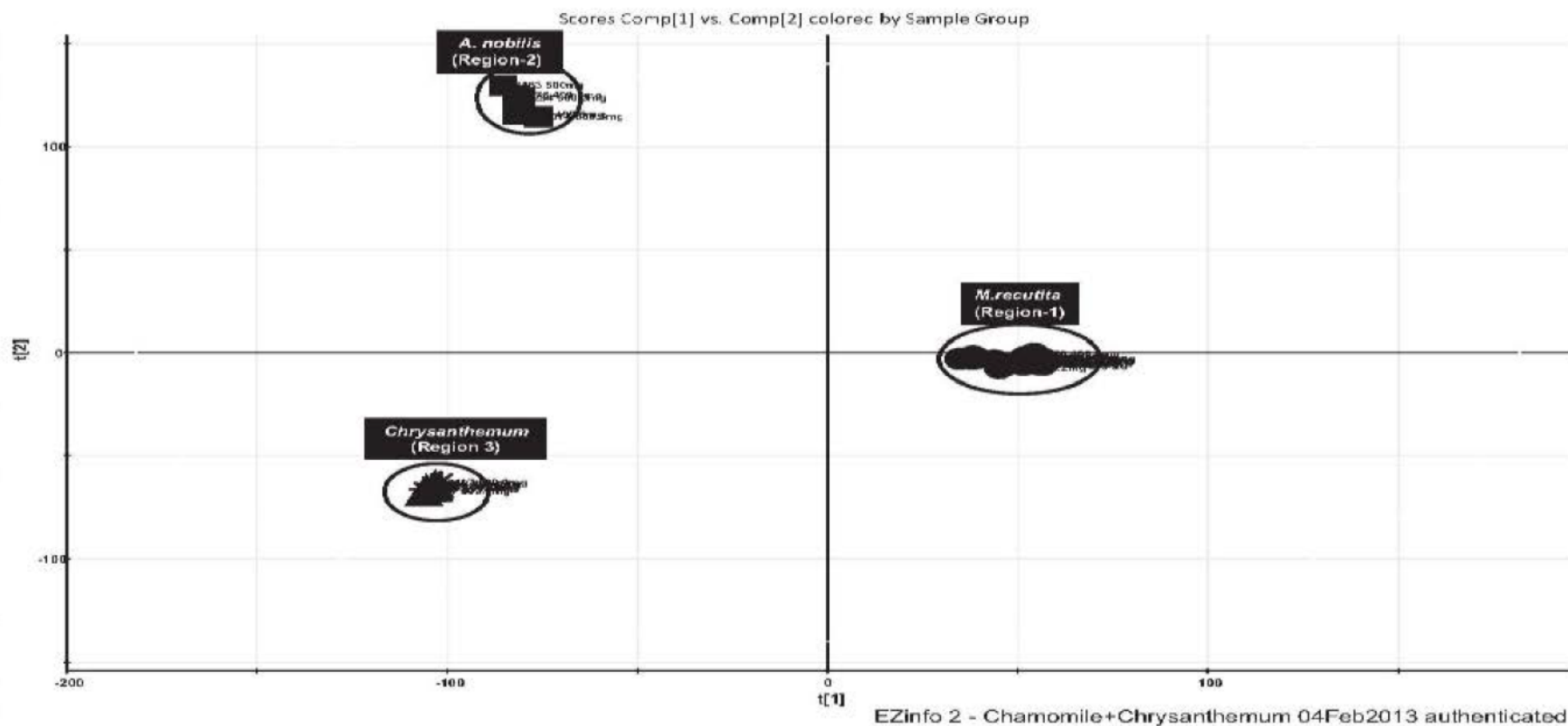
Juhua

Reference standards (Rf order): rutin (1), luteolin-7-O-glucoside (2), chamaemeloside (3), apigenin-7-O-glucoside (4), luteolin (5), apigenin (6), and umbelliferone (7)



PCA score plots for authenticated chamomile samples and *Chrysanthemum*

LC-MS Used in Natural Products/Dietary Supplements



LC-MS Used in Natural Products/Dietary Supplements

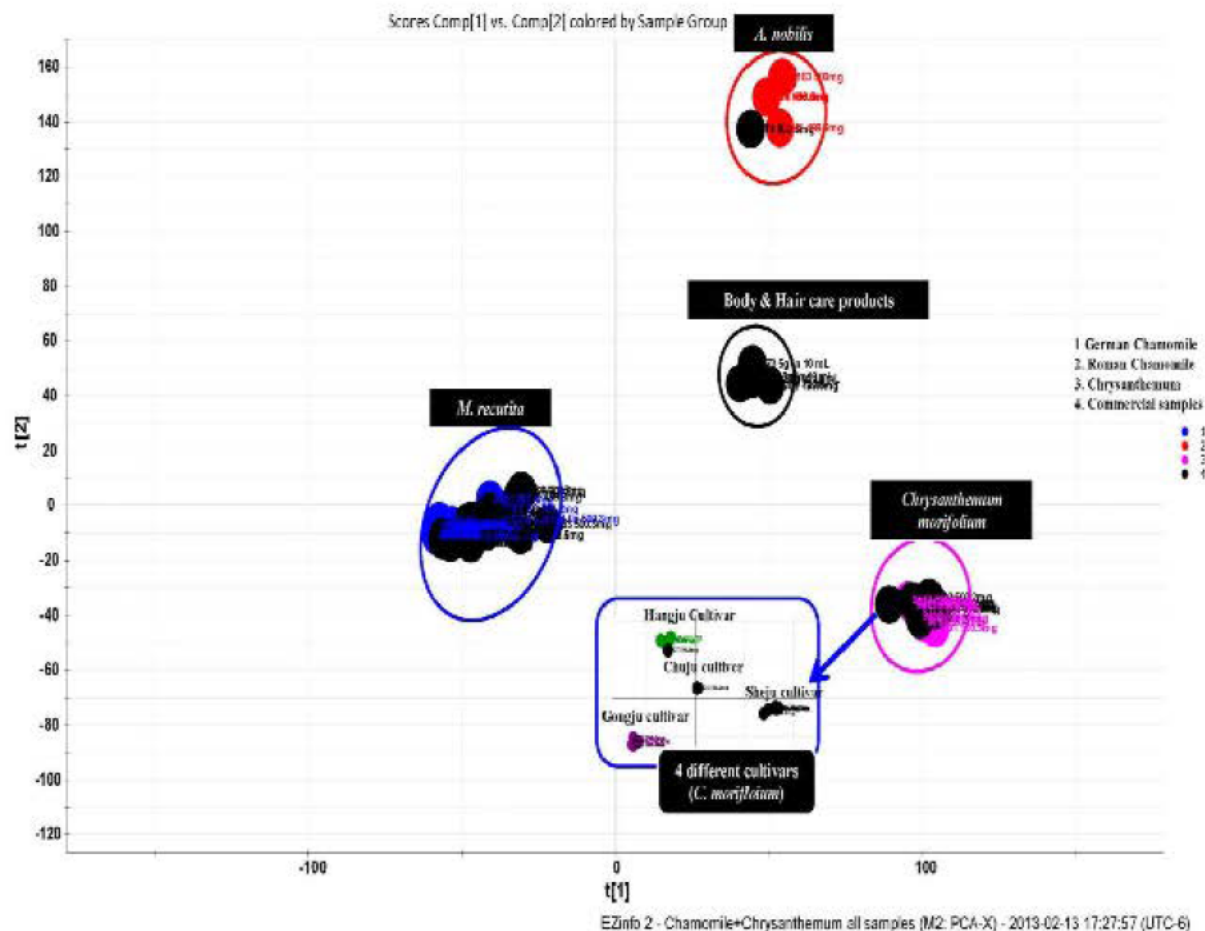


Fig. 4. Chamomile/Chrysanthemum authenticated and commercial samples: PLS-DA scores plot of first and second principal component on the basis of UPLC/Q-TOF-MS data.

Skin Sensitization

- Major concerns:
 - Chemicals present in consumer products and workplace
 - Widespread usage of plant extracts in cosmetic products
 - Animal-Based Tests** required by current regulatory guidelines:
 - a) Local Lymph Node Assay
 - b) Guinea Pig Maximization Test
- Need for reliable alternative methods for preliminary screening of potential sensitizers to limit use of animal tests
- Non-animal based chemical reactivity (in chemico) methods are proposed in order to understand the chemical and biological aspects of skin sensitization.

Skin Sensitization: Non-Animal models

- Spectrophotometric assay to detect the reactivity of electrophilic compounds to glutathione (GSH).¹
- High Throughput Screening: Peptide depletion assay with GSH or other synthetic peptides using HPLC-UV.^{2,3}
- Quantitative LC-MS method to characterize adduct formations using a heptapeptide assay (DPRA).⁴
- Kinetic spectrophotometric assay using nitrobenzenthionol as an electrophilic trap using stop-flow techniques and UV detection.⁵
- KeratinoSenseTM: is based on a stable reporter construct consisting of a luciferase gene under the control of the antioxidant response element (ARE) in a HaCaT (immortalized keratinocyte) cell line.

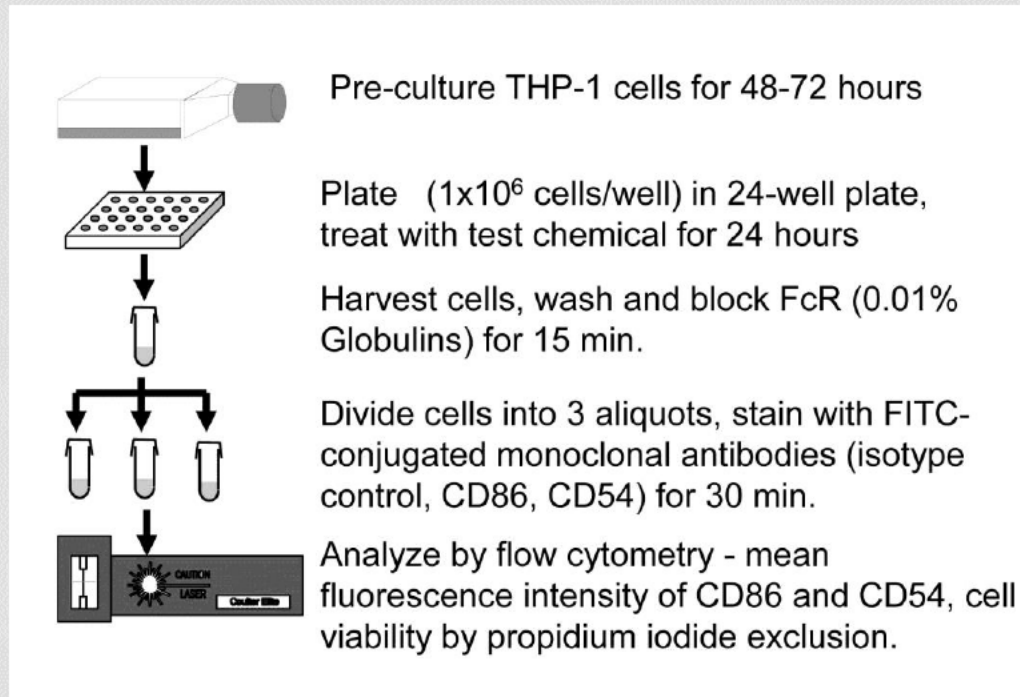
¹SAR QSAR Environ. Res 16(4), 313-322, 2005. ²Toxicol. Sci 81(2), 332-343, 2004. ³Toxicol. Sci 97(2), 417-427, 2007.

⁴Toxicol. Sci 106(2), 464-478, 2008. ⁵Chem. Res. Toxicol. 23(5), 918-925, 2010



Human Cell Line Activation Test (h-CLAT)

- Human THP-1 cell-line activation is used as a measure for skin sensitization and part of an integrated test strategy.
- Expression of two cell surface antigens, CD86 and CD54, is measured by specific antibody staining and subsequent detection by flow cytometry.
- Two of three independent measurements at any dose should exceed the positive criteria (CD86 >150% or CD54 >200%) in order to be judged as positive.



Determination of Sensitization Agents Within Chamomile Species

EC1.5 and IC₅₀ Summary Table

Assay Date	IVS Test Article	Sponsor Designation	EC 1.5 value (µg/mL)	Mean IC ₅₀ (µg/mL)		Potential Sensitizer?
				MTT	NRU	
	11AH38	German Chamomile Hexane Fraction (9172 Hexane, JZ-11A-2-2)	0.67	168	160	YES
	11AH39	German Chamomile Chloroform Fraction (9172 CHCl ₃ , JZ-11A-13-2)	5.85	155	72.6	YES
	11AH40	German Chamomile Ethanol extract (3763 EtOH, IKX-1-55.11)	21.23	>400	>400	YES
26 July 2011	11AH41	Roman Chamomile Hexane Fraction (9254 Hexane, JZ-11A-13-2)	2.56	88.5	81.4	YES
	11AH42	Roman Chamomile Chloroform Fraction (9254 Hexane, JZ-11A-13-3)	0.50	9.96	8.10	YES
	11AH43	Chamomile essential oil (9569)	2.23	9.47	9.44	YES
	11AH44	Bisabolol	>400	9.38	9.30	NO
7 Sept 2011	Chloroform	Chloroform	>400	>400	>400	NO
	Hexane	Hexane	>400	>400	>400	NO
26 July 2011	Cinnamic Aldehyde	Positive Control	10.26 µM	> 64 µM	> 64 µM	YES
7 Sept 2011			8.76 µM	> 64 µM	> 64 µM	YES

EC1.5 and IC₅₀ Summary Table

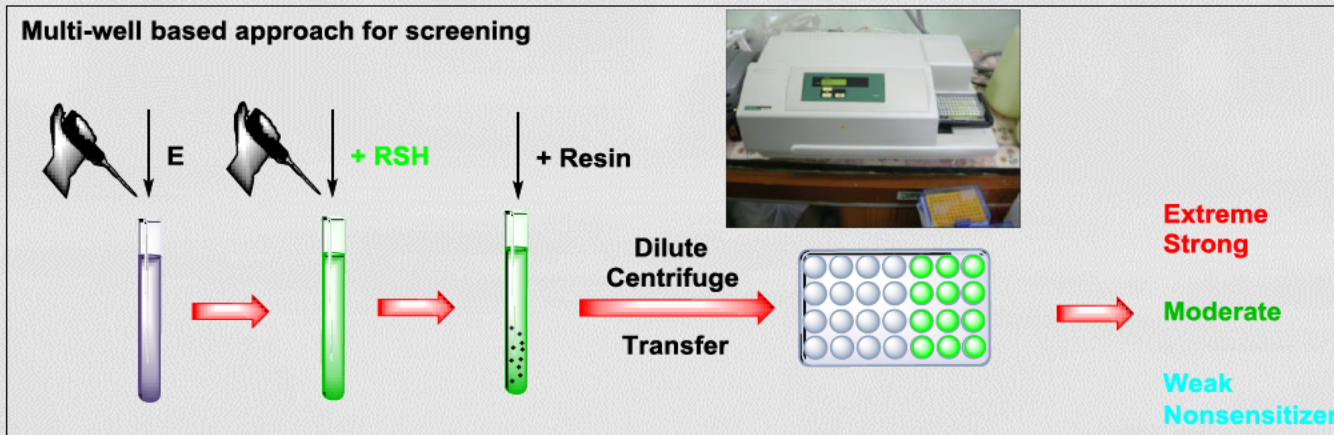
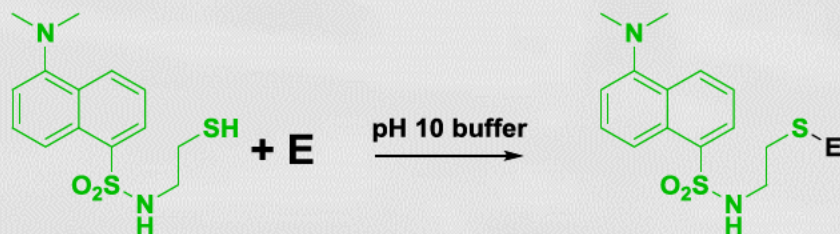
Assay Date	IVS Test Article Number	Sponsor's Designation	EC 1.5 value (µg/mL)	Mean IC ₅₀ (µg/mL)		Potential Sensitizer?
				MTT	NRU	
	12AE99	JZ-12-11-3, German Chamomile Extract	3.29	>400	377	YES
	12AF00	JZ-12-15-1, German Chamomile Fraction	2.96	291	352	YES
	12AF01	JZ-12-14-3, German Chamomile Fraction	3.02	336	>400	YES
	12AF02	JZ-12-14-4, German Chamomile Fraction	0.471	>400	>400	YES
15 May 2012	12AF03	JZ-12-14-5, German Chamomile Fraction	24.8	279	290	YES
	12AF04	JZ-12-14-6, German Chamomile Fraction	34.8	>400	>400	YES
	12AF05	JZ-12-7-3, Roman Chamomile Extract	0.7.8	83.5	80.7	YES
	Cinnamic Aldehyde	Positive Control	13.3	>64	>64	YES

Determination of Sensitization Agents Within Chamomile Species

- German and Roman Chamomile extracts along with fractions were evaluated for sensitization potential using KeratinoSens assay by a commercial laboratory (IIVS).
- Several fractions are found to have sensitization potential without any cytotoxicity.
- Direct Peptide Reactivity Assay failed to estimate the sensitization potential of the pure compounds of German Chamomile due to their solubility in only DMSO. The solvent, DMSO was found to have detrimental effects on DPRA.

Non-Animal models- HTS Method

Tools for estimating the skin sensitization potential of botanical ingredients/extracts



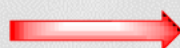
Non-Animal models- NMR Method

Tools for estimating the skin sensitization potential of botanical ingredients/extracts

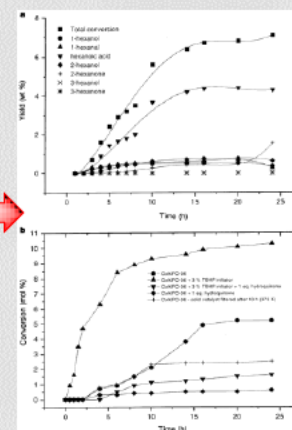
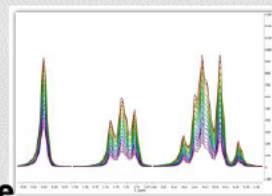
NMR based approach for kinetic and mechanistic details



Mix RSH, E and base
in deuteriated solvent



Acquire multiple
spectra over a 3h
period



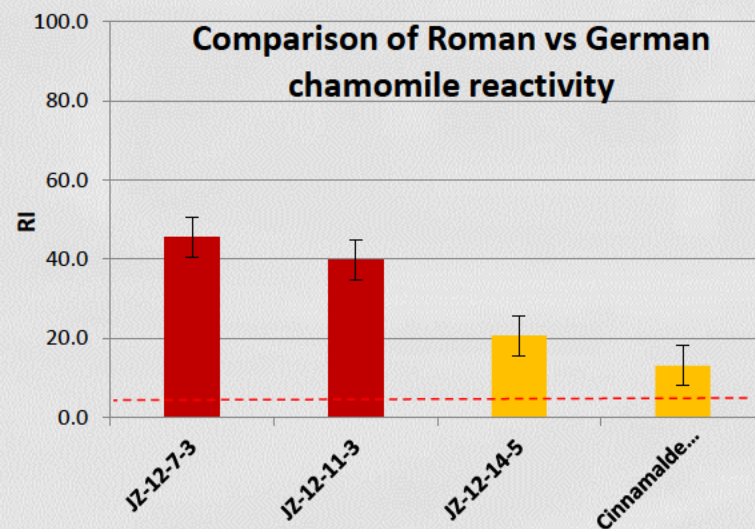
HTS Method on Roman & German

- Samples of authenticated German (*Matricaria recutita*) and Roman chamomile (*Chamaemelum nobile*) previously evaluated *in vitro* using Keratinosens¹ were screened using the newly developed DCYA-HTS
- Roman chamomile (RC) crude extract resulted in a stronger response compared to German chamomile (GC) extract and enriched fractions
- The results obtained with the fluorescence assay were comparable to *in vitro* results

Sample	Description	HTS	Keratinosens ¹
		RI*	IC ₅₀
JZ-12-7-3	Roman Chamomile Extract	45.5	0.718
JZ-12-11-3	German Chamomile Extract	39.8	3.29
JZ-12-14-5	German Chamomile Fraction	20.6	24.8
Cinnamaldehyde	Positive control	14.9	13.3

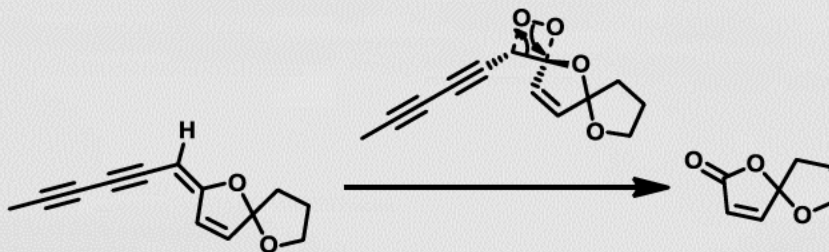
*Note: for HTS results, the higher the RI, the stronger the sensitizer

¹See Report Jul 31st 2013 study number 12AE99-AF05.170000, Institute for In Vitro Sciences,



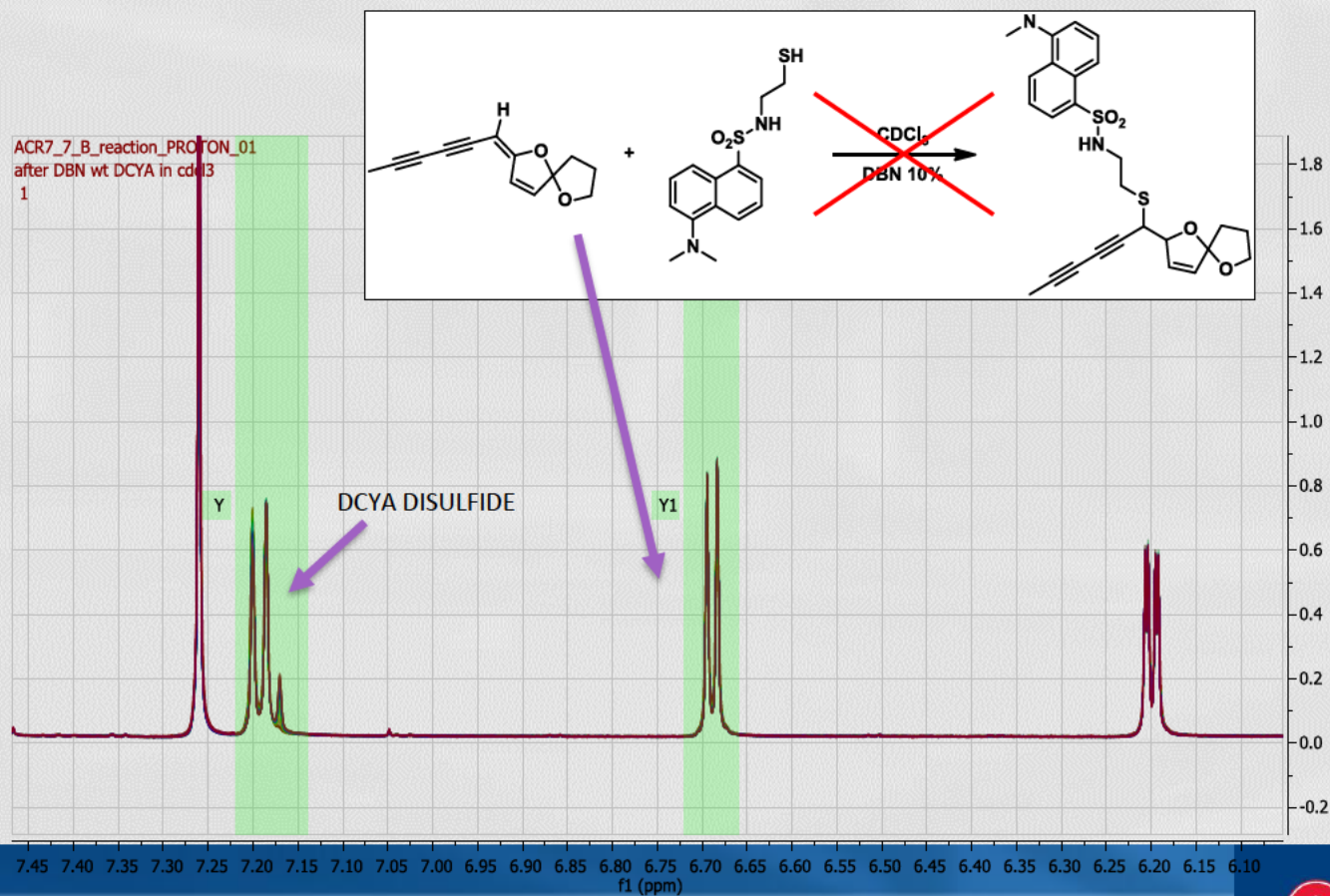
Sensitization potential of Tonghaosu

- The polyacetylene **tonghaosu** is one of the main components of **German chamomile**
- The compound can be considered as a potential hazard because it contains structural alert moieties and due to its structural **resemblance to known sensitizers** (e.g. falcarinol)¹
- kNMR and HTS studies were performed but the compound was found to be **not reactive**
- A high **instability** of the compound was observed, which led to the isolation of a degradation product identified as dioxo-spiro lactone.



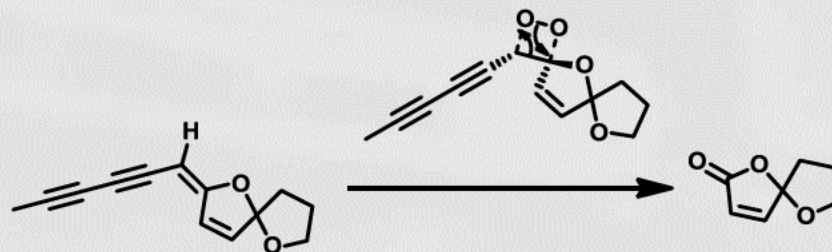
SENSITIZATION POTENTIAL OF TONGHAOSU (2)

Both pure and enriched fractions were classified non- to weak sensitizers

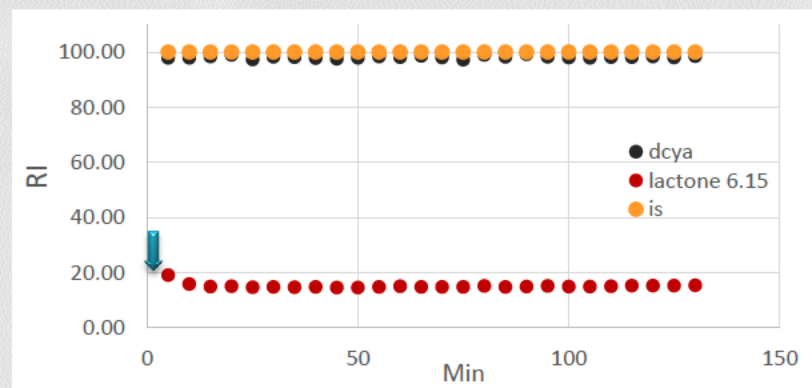
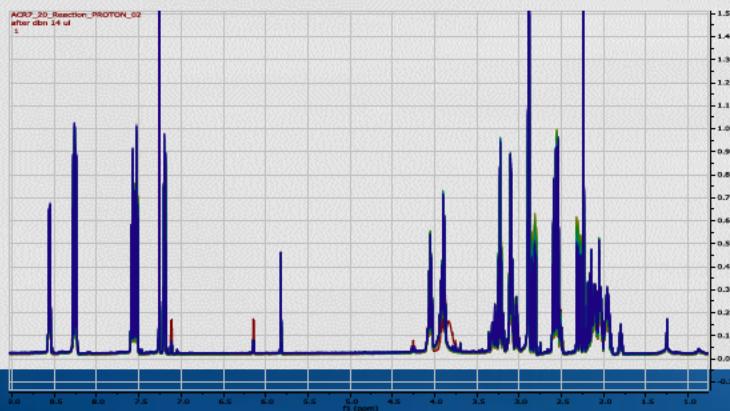


SENSITIZATION POTENTIAL OF TONGHAOSU (4)

After structural elucidation and synthesis, the impurity was identified as a small dioxaspiro derivative containing a Michael acceptor moiety.

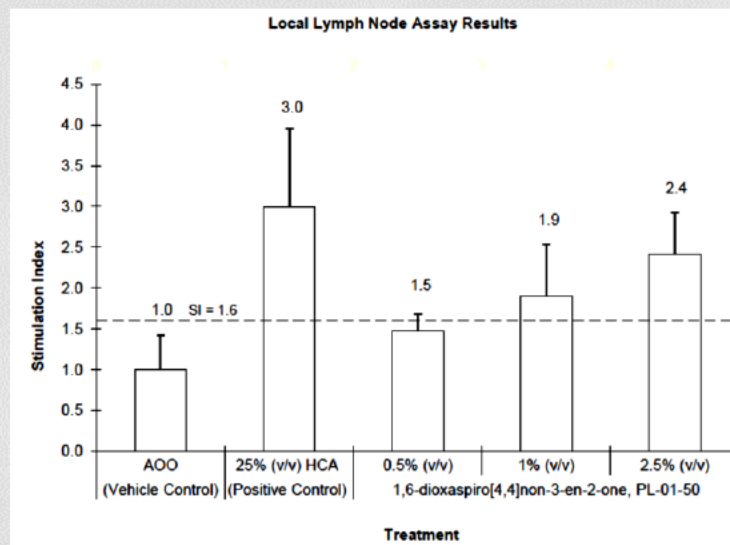


The compound was evaluated for its sensitization using kNMR and resulted in 83% reaction with DCYA. The compound was thus classified as moderate-strong sensitizer.



LLNA data

- In order to confirm the sensitization potential of the identified lactone, the compound synthesis was scaled-up to meet the amount requirement for LLNA, which was performed by an external laboratory¹
- The compound was tested at different concentrations, and classified as a dermal sensitizer



Outcome

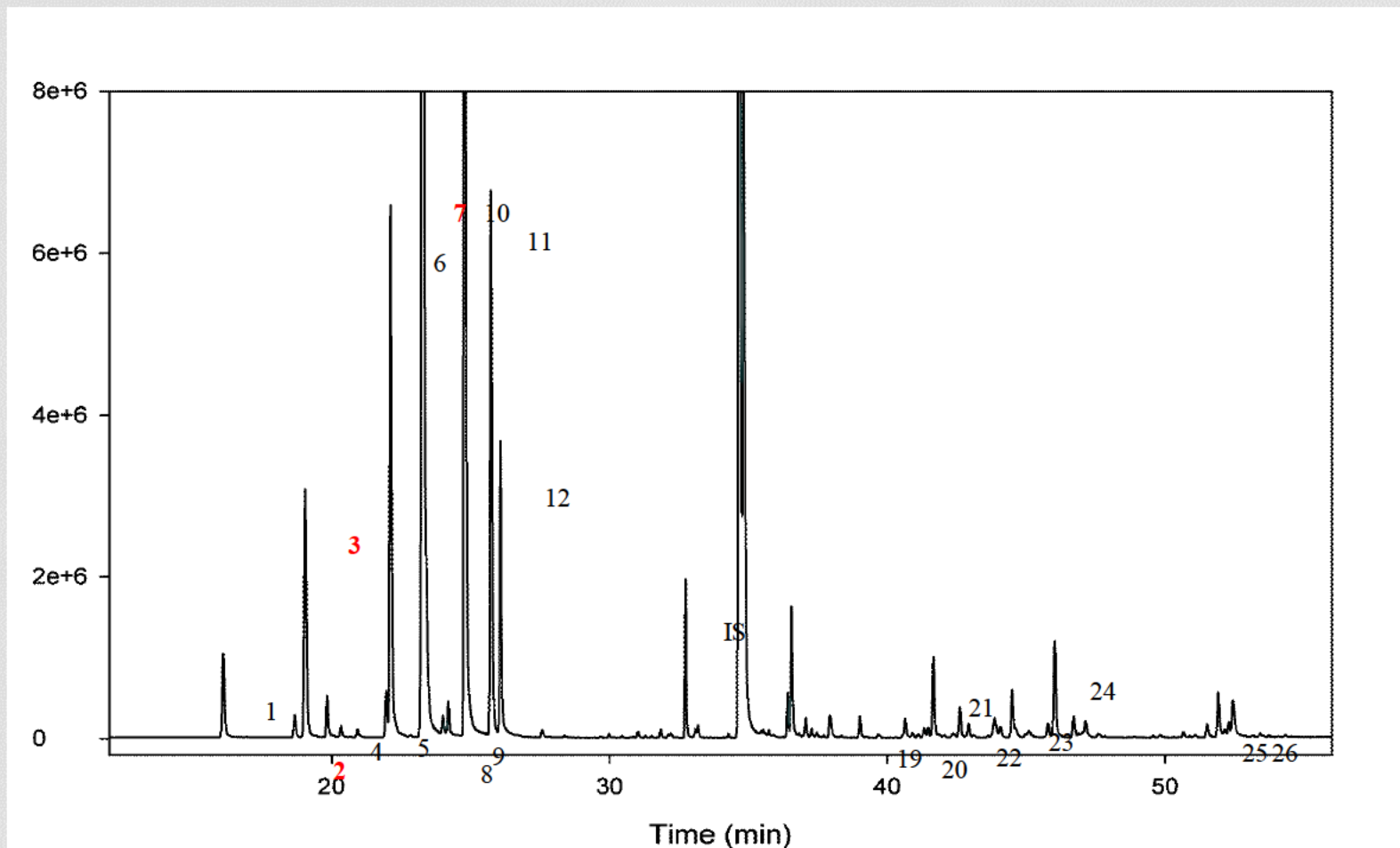
- According to WHO monogram on German chamomile, very few cases of allergy were specifically attributed to German chamomile.¹ Adverse effects were attributed to presence of Lactones!!!
- Six chemo-types of German chamomiles (variation in distribution of chamazulene, bisabolane sesquiterpenes, Tonghaosu and apigenin content).
- Tonghaosu is one of the major marker compound in essential oil of German chamomile
- 130-160mg/g of Tonghaosu in EO of authentic German chamomile (NPC-9850)
- Several commercial EOs of German chamomiles were studied and found 50-160mg of Tonghaosu per gram of EO

¹Hausen BM, Busker E, Carle R. Über das Sensibilisierungsvermögen von Compositenarten. VII. Experimentelle Untersuchungen mit Auszügen und Inhaltsstoffen von *Chamomilla recutita* (L.) Rauschert und *Anthemis cotula* L. *Planta medica*, 1984:229–234.

Tea Tree Oil

- Essential oil obtained by steam distillation of the foliage and terminal branchlets of *Melaleuca alternifolia*, *Melaleuca linariifolia*, *Melaleuca dissitiflora* or other species of the genus *Melaleuca*
- Due to the increasing market for TTO, there is a growing trend toward adulteration or substitution of these products
- A total of 104 tea tree oil samples were provided by:
Attia Ltd
P. O. BOX 903
Casino NSW 2470
Australia
- Sample Preparation: 5 μ L of each sample was dissolved in 1 mL n-hexane. The internal standard of n-tridecane was applied to all the solutions with a fixed concentration. Duplicated injections were made for the chiral GC analysis.

Typical Chiral Chromatogram of *M. alternifolia*



Enantiomeric compounds were marked in red.

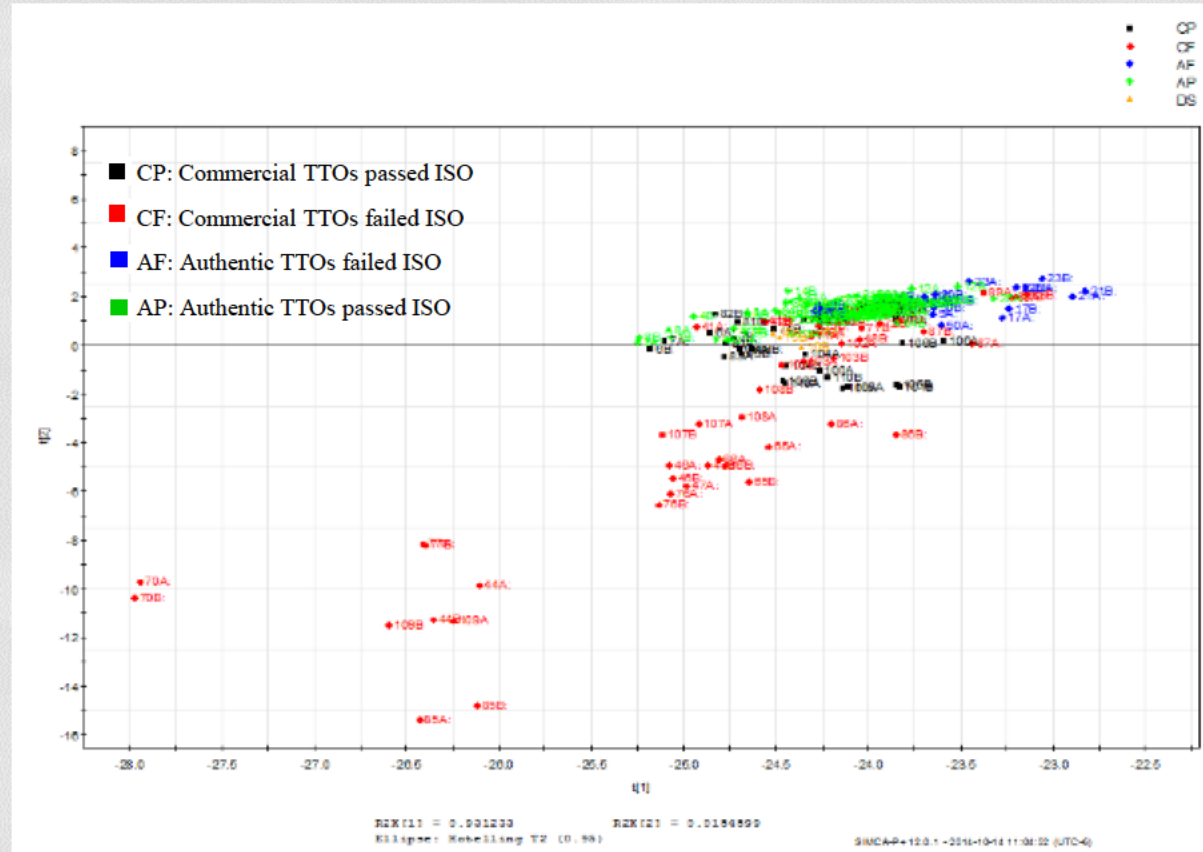
Identification of the Major Compounds in Tea Tree Oil

Peak No.	Compound	t _R	%
1	α -thujene	16.10	1.01
2	(-)- α -pinene	18.67	0.27
3	(+)- α -pinene	19.05	3.02
4	β -myrcene	19.85	0.44
5	β -pinene	21.97	0.52
6	α -terpinene	22.12	5.83
7	p -cymene, (\pm) limonene	23.26	25.07
8	(\pm)- β -phellandrene	24.01	0.21
9	(\pm)- β -phellandrene	24.19	0.37
10	γ -terpinene	24.78	17.27
11	α -terpinolene	25.73	5.61
12	1,8-cineole	26.07	2.88
13	(+)-terpinen-4-ol	34.64	16.35
14	(-)-terpinen-4-ol	34.53	9.36
15	(-)- α -terpineol	36.41	0.36
16	(+)- α -terpineol	36.56	1.25
17	α -copaene	37.07	0.20
18	p -cymene-8-ol	37.93	0.29
19	α -gurjunene	39.03	0.20
20	β -caryophyllene	40.65	0.25
21	alloaromadendrene	41.66	0.85
22	aromadendrene	42.61	0.32
23	viridiflorene	44.50	0.69
24	δ -cadinene	46.02	1.19
25	globulol	51.92	0.51
26	viridiflorol	52.45	0.58
Total			94.87



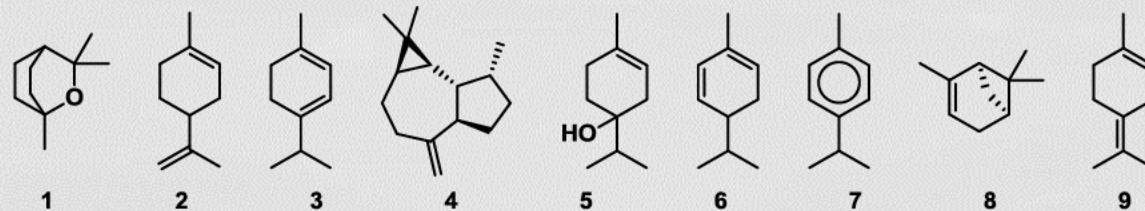
PCA for authentication of TTO

- All the authentic TTOs that met the ISO standards cluster in a single group (green) in the PCA score plot.
- The chiral ratios of α -pinene, limonene, terpinen-4-ol and α -terpineol for the tea tree oil samples in this group were also within the ranges of norms.



Sensitization Potential of TTO

- Tea tree oil is a popular remedy for eczema, acne, skin infections, wounds, burns, insect bites and mycosis
- The fresh oil has been classified by LLNA as a mild sensitizer, while aged TTO samples have been found to be stronger sensitizers than fresh oils.
- Several terpenoids (**1-9**) have been proposed as candidate sensitizers. [1,2] These compounds do not contain mechanistic domain to be regarded as a potential hazard.
- It has been previously hypothesized that oxidization products formed during oil aging can be responsible for the increased allergenic potential.[3]

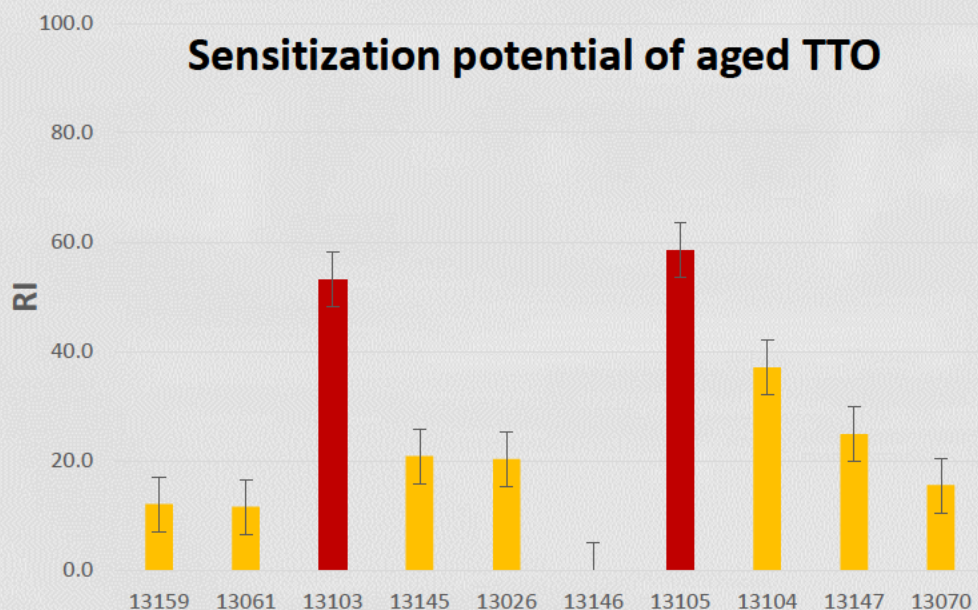


Sensitization Potential of TTO

- Ten samples of TTO and related species previously analyzed by our group were used for the aging study
- Aged samples were then characterized by GC-MS and compared to the original chemical composition before aging
- The content of p-cymene and hydrogen peroxide were quantified as indication of aging
- The aged oils were then tested for their sensitization potential using the DCYA-HTS assay
- The principal constituents of TTO were also investigated for their sensitization potential after accelerate aging conditions

Sensitization Potential of TTO

- From the DCYA-HTS results, some oils scored higher in the sensitization potential
- With regard to authentic TTO (*Melaleuca alternifolia* and *M. linariifolia*), a correlation between content of *p*-cymene and sensitization potential was found but not a clear correlation with the peroxide content

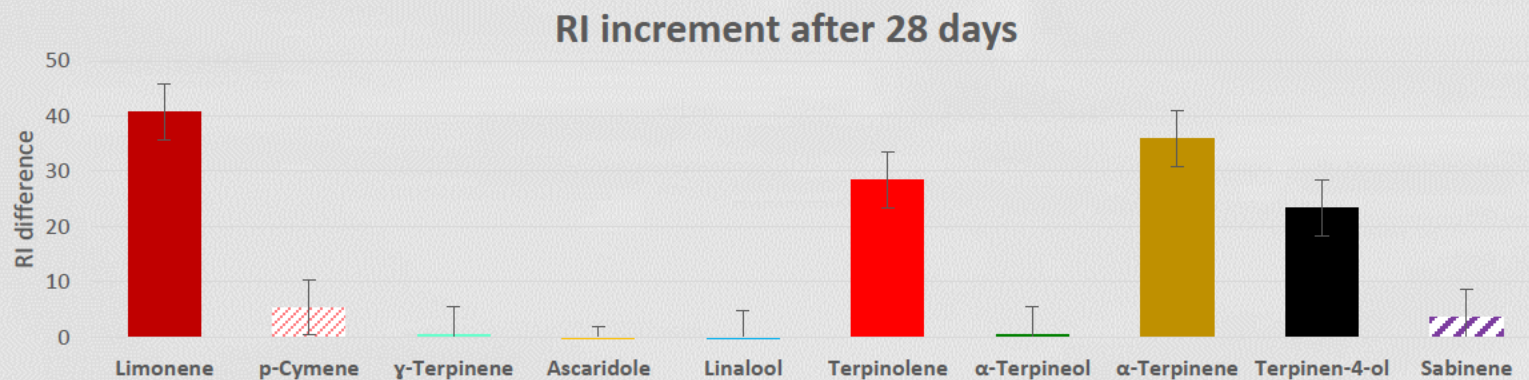
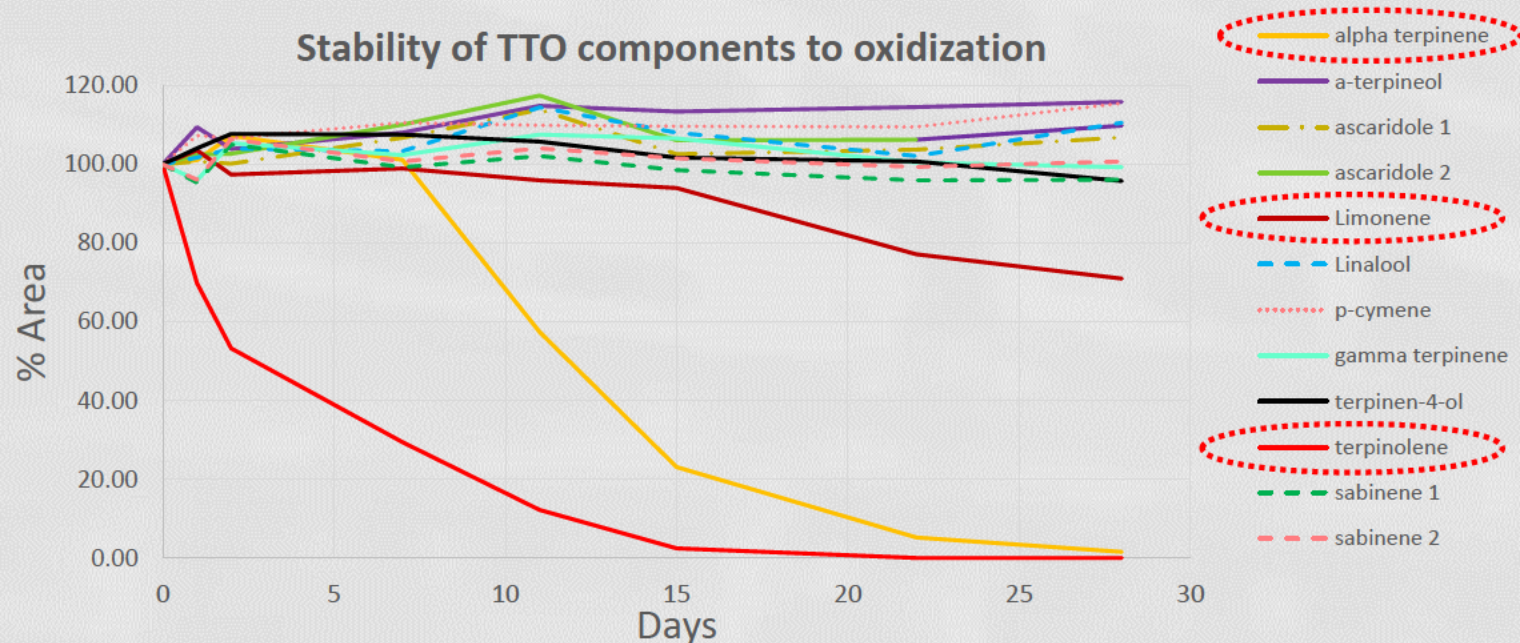


	PLANT SOURCE
# 13159	Melaleuca alternifolia
# 13061	Melaleuca cajuputi
# 13103	Melaleuca leucadendron
# 13145	Melaleuca
# 13026	Melaleuca linariifolia
# 13146	Leptospermum scoparium
# 13105	Melaleuca alternifolia
# 13104	Melaleuca viridiflora
# 13147	Kunzea ericoides
# 13070	Leptospermum + Melaleuca

Sensitization Potential of TTO

- As a clear correlation between the monoterpenes content, aging and potential sensitization was found, the principal TTO components were also investigated for their sensitization potential
- The pure compounds were aged by bubbling oxygen continuously for 28 days.
- Fresh and aged compounds were analyzed for their stability over time by GC-MS and for their potential reactivity using HTS
- **Terpinolene, α -terpinene and limonene were found unstable under oxidization conditions**

Sensitization Potential of TTO



Sensitization Potential of TTO

CONCLUSIONS

- Ten TTOs and related species were investigated for their sensitization potential in relation to the aging process for the oil
- The HTS-DCYA proved to be a very useful tool to analyze the sensitization potential of complex essential oils
- A correlation between aging and sensitization potential of genuine TTOs was found
- The major components from TTO were also analyzed individually for their sensitization potential
- Non-aged pure compounds were found non- to weakly reactive
- When aged under accelerated oxidization conditions, a correlation between the degradation of the compounds and their increased sensitization potential was found
- The obtained results are in agreement with known observations from the literature with regard to the oxidization of TTO as well as individual monoterpenes as a potential sources of skin sensitization

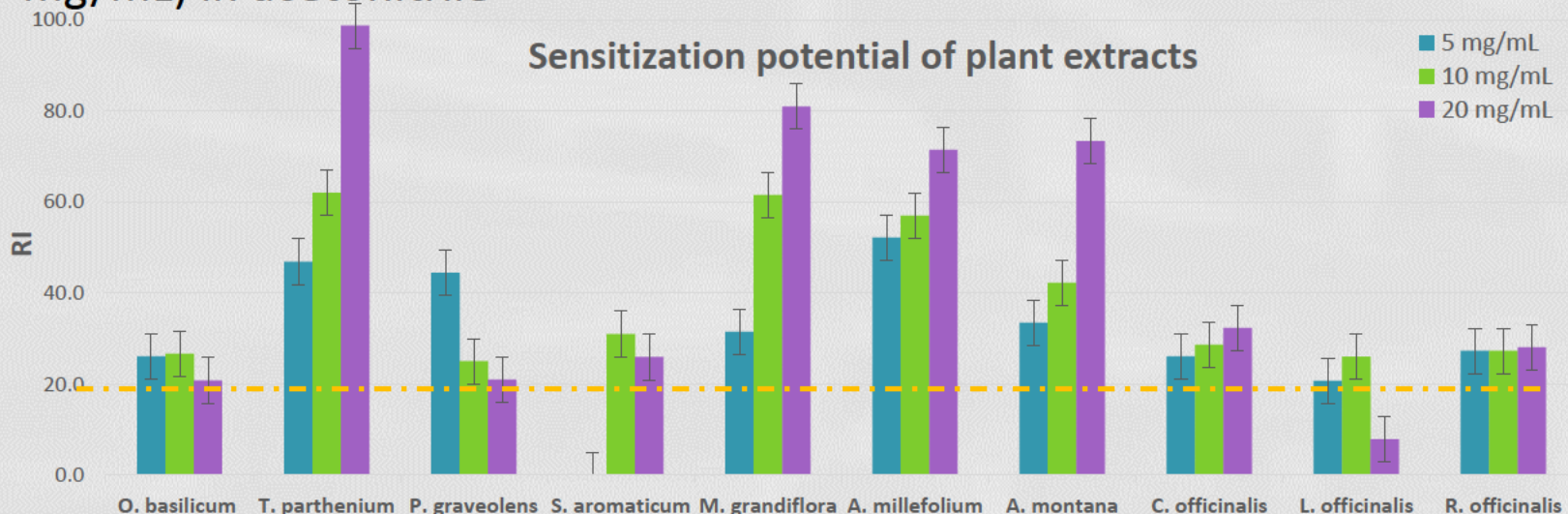
Systematic Investigation of Plant Extracts

Using HTS-DCYA

- A validation of the HTS-DCYA assay of complex crude mixtures is currently ongoing
- A standard extraction procedure has been optimized to rapidly test plant extracts for the presence of both volatile (EO) and non-volatile potential sensitizers
- Fifty plants of interest for cosmetic and fragrances use have been selected
- The selection included several plant parts (barks, leaves, flowers, roots, fruits, gums and resins) to identify potential sources of interferences with the fluorescence response

Plant Extracts and HTS-DCYA

- Methanol extracts were tested at different concentrations (5, 10 and 20 mg/mL) in acetonitrile



Sample	Genus	Species	Part	NCNPR#	Reported sensitization
1	<i>Ocimum</i>	<i>basilicum</i>	leaf	3635	LLNA - Moderate* ¹
2	<i>Tanacetum</i>	<i>parthenium</i>	mixed parts	1	Human sensitizer ²
3	<i>Pelargonium</i>	<i>graveolens</i>	aerial parts	9801	LLNA Extremely weak ¹
4	<i>Syzygium</i>	<i>aromaticum</i>	Flower-bud	4962	LLNA Weak ¹
5	<i>Magnolia</i>	<i>grandiflora</i>	leaf	na	Human sensitizer ³
6	<i>Achillea</i>	<i>millefolium</i>	flower	3552	Human sensitizer ⁴
7	<i>Arnica</i>	<i>montana</i>	flower	8350	Human sensitizer ⁶
8	<i>Calendula</i>	<i>officinalis</i>	Flower	3668	Rare in human ⁴
9	<i>Lavandula</i>	<i>officinalis</i>	Flower	3671	Sensitizer after aging of the EO* ⁵
10	<i>Rosmarinus</i>	<i>officinalis</i>	Leaf	5956	Potential sensitizer in human*

*Used as essential oil, suspected aged-related sensitization

1Lalko, J. and Api. A. M. Food chem toxicol 44, 2006, 739-746. 2 Paulsen, E. et al. Contact dermatitis 63, 2010, 146-150. 3Guin, J. D. et al. Dermatologic clinics 8, 1990, 81-84. 4Calapai, G. et al. Contact dermatitis 71, 2014, 1-12. 5Gangemi, S. et al. Contact dermatitis 72, 2015, 193-205. 6Paulsen, E. Contact Dermatitis 47, 2002, 189-198.

Plant Extracts using HTS-DCYA

- Preliminary results on a systematic analysis of plant extracts have been validated using plants previously classified by LLNA or from clinical data
- The use of a minimum of 3 concentrations was chosen to minimize the potential interference from non specific response caused by the presence of chlorophylls, pigments, etc...
- The concentration-dependent response for plants known for their content of potential allergens (*T. parthenium*, *A. millefolium*, *M. grandiflora* and *A. montana*) was on average 3 to 5 times higher than plants with few reported cases of skin allergy

List of current 26 allergens

- Amyl Cinnamal
- Amylcinnamyl Alcohol
- Anise Alcohol
- Benzyl Alcohol
- Benzyl Benzoate
- Benzyl Cinnamate
- Benzyl Salicylate
- Butylphenyl Methylpropional
- Cinnamyl Alcohol
- Citral
- Citronellol
- Coumarin
- Eugenol
- Farnesol
- Geraniol
- Hexyl Cinnamal
- Hydroxyisohexyl 3-cyclohexene Carboxaldehyde
- Hydroxycitronellal
- Isoeugenol
- Alpha-isomethyl Ionone
- Limonene
- Linalool
- Methyl 2-Octynoate
- Oak Moss Extract
- TreeMoss Extract
- Cinnamal

Classification of 24 fragrances in non-animal methods

Pre/pro-haptens				Potentially reactive compounds			
Compound	LLNA (EC3 %) ^a	Keratinosens EC3 [μM] ^b	Cys-DPRA (% remaining) ^b	Compound	LLNA (EC3 %) ^a	Keratinosens EC3 [μM] ^b	Cys-DPRA (% remaining) ^b
Amylcinnamyl Alcohol	NC		Non reactive ^c	Amyl Cinnamal	7.6-11.2 (Weak)	>2000	99.4
Anisyl alcohol	5.9			Benzyl Cinnamate	18.4		
Benzyl Alcohol	NC			Citral ¹	1.2-13		
Benzyl Benzoate	17.0-18.4 (Weak)	142.47	99.8	Cinnamal ¹	0.2-3.1 (Moder)	63.94	29.4
Benzyl Salicylate	2.9			Coumarin ¹	19.2-45.7 (Non)	479.96	99.0
Cinnamyl alcohol ³	19.1-21 (Weak)	>2000	100.0	Hexyl Cinnamal	4.4-17.6 (Weak)	>2000	100.0
Citronellol ²	43.5			Alpha-isomethyl Ionone	21.8		
Eugenol ³	5.8-40.9 (Weak)	>2000	90.8	Methyl 2-Octynoate	0.5		
Farnesol	4.1-5.5						
Geraniol ^{2,3}	11.4-57(Weak)	>2000	100				
Hydroxycitronellal	18.8-33.0	142.91	82.5				
Lylal	17 (Weak)	197.12	60.6				
Isoeugenol ³	0.5-5 (Moder)	259.43	10.7				
Lilial	2.9-18.7 (Weak)	>2000	86.0				
Limonene ^{1, 2}	10-70 (Weak)		Non reactive ^c				
Linalool ^{1, 2}	55 (Weak)	>2000	100				

¹Already tested using HTS-DCYA assay ²Reported data suggest chemical transformation ³Reported data suggest metabolic transformation

^ahttp://ntp.niehs.nih.gov/iccvm/docs/immunotox_docs/llna-pot/3b-appc-brd-annexii-http://ntp.niehs.nih.gov/iccvm/docs/immunotox_docs/llna-pot/3b-appc-brd-annexii-1.pdf1.pdf ^bNatsch, Andreas, et al. *Journal of Applied Toxicology* 33.11 (2013): 1337-1352. ^c<https://eurl-ecvam.jrc.ec.europa.eu/eurl-ecvam-recommendations/files-dpra/DPRA%20Validation%20Study%20Report.pdf>



Conclusions

- Skin sensitization is an important toxicological end-point for risk assessment of chemicals
- Chemical approaches can greatly contribute to the assessment of the Adverse Outcome Pathway (AOP) as exemplified by the validation of the DPRA as an alternative method to animal tests
- Plants and natural extracts are gaining positive acceptance among consumers in the cosmetic market, but they may also represent a source of potentially hazardous compounds
- It is thus vital to be able to recognize and characterize the hazard of complex mixtures or challenging cases (e.g. essential oils)
- The two methods recently developed at UM enable a fast and inexpensive screening and have been successfully tested in a number of challenging cases in the realm of plant extracts

Conclusions

- As no alternative method has been successfully validated for mixtures, *in vivo* assays (LLNA) on plant extracts can pose an ethical and economical drawback as exemplified by the fact that very few plants have been investigated by LLNA and the majority of reported adverse effects were primarily from clinical studies
- Due to the extreme complexity and dynamics of the natural products, the lack of adequate versatile, economical and ethical approaches presents an urgent need in the risk assessment of botanicals used in cosmetic and fragrances
- Both the DCYA-HTS and kNMR can be used to discriminate between risk potential of different plants (e.g. chamomile varieties), essential oils (tea tree) and how their activities vary with storage conditions and changes over time (tonghaosu, TTO) and quickly identify potential risk when exploring new plants as sources of topically applied formulations

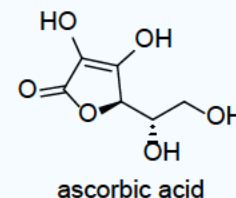
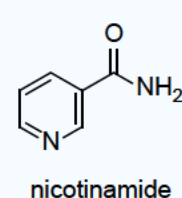
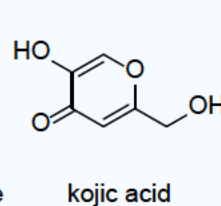
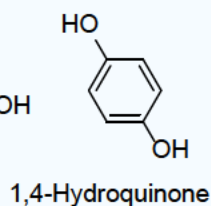
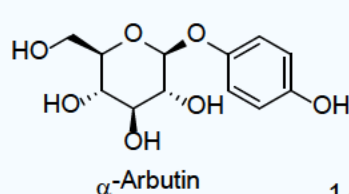
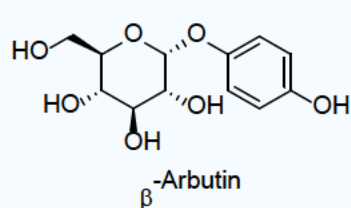
תודה
Dankie Gracias
Спасибо شكرياً
Merci Takk
Köszönjük Terima kasih
Grazie Dziękujemy Děkojame
Ďakujeme Vielen Dank Paldies
Kiitos Tänname teid 谢谢
Thank You Tak
感謝您 Obrigado Teşekkür Ederiz
감사합니다
Σας ευχαριστούμε ขอบคุณ
Bedankt Děkuje vám
ありがとうございます
Tack



Synopsis on OCAC's projects

ARBUTIN

- ❖ Arbutin is the glycoside derivative of hydroquinone. The β -glycoside anomer is commonly found in species of several plant families in nature, whereas commercial α -arbutin is usually obtained by biotransformation or by chemical synthesis
- ❖ Both β - and α -arbutin became popular skin whitening agents because of their ability to interfere with the melanin synthesis
- ❖ Because of their structural similarity to hydroquinone, both arbutins can be regarded as potential sources of the compound of concern hydroquinone upon topical application
- ❖ Other popular “natural” whitening agents include kojic acid, nicotinamide, ascorbic acid and resorcinol



TASKS

✓ **Task #1:**

Natural occurrence of arbutins in plant species

✓ **Task #2:**

Inter-conversion and stability (chemical and *ex vivo*)

✓ **Task #3:**

Distribution in cosmetics including the development of an LC method for detecting arbutin and commonly found whitening ingredients

Natural occurrence of arbutins in plant species

➤ **Aim:**

Investigation of distribution of β - and α -arbutin in plants

➤ **Method developed:**

- 1) Extraction procedure for the analysis of arbutins in plants
- 2) HPLC-UV method

➤ **Experiments performed:**

HPLC screening

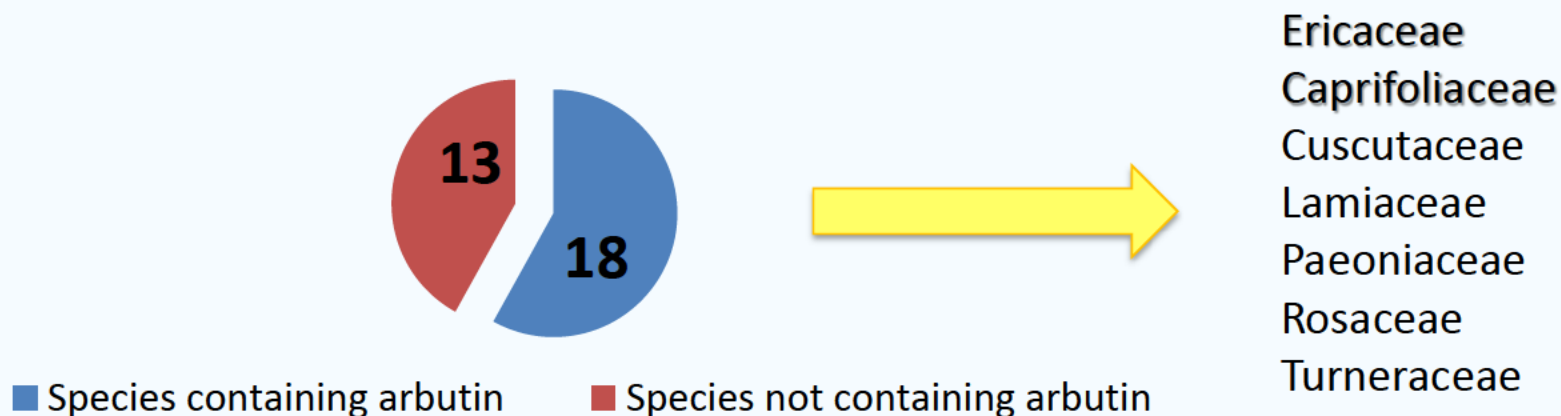
TASK #1



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MISSISSIPPI
National Center for
Natural Products Research

➤ Results:

A total of 59 specimens covering 31 species, (22 genera and 19 families) were analyzed for the occurrence of both arbutins in different part plants



➤ Conclusions:

Eighteen species were found to contain β -arbutin, while none was found as positive source of α -arbutin

Chemical and enzymatic stability of arbutins

➤ **Aim:**

Assessing the stability of arbutins with regard to the potential release of hydroquinone or epimerization in conditions of potential relevance for cosmetics

➤ **Experiments performed:**

HPLC screening

Nuclear Magnetic Resonance (NMR)

Optical Rotation (OR)

Experimental procedures

- Sixteen months stability study on 9 commercial arbutins preparations (sera, creams, lotions and gels) with different formulation and pH characteristics (method used: HPLC-UV)
- NMR/OR studies for stability of arbutins in solution in absence of additives or buffers
- Metabolic stability studies using pear peels as surrogate for skin metabolism and HPLC-UV method

Results

- The stability of both arbutins was lower in liquid preparations. The less stable formulation was a serum (pH 8.8), resulting in 20% loss of β -arbutin after 16 months
- Creams and semi-solid formulations were relatively stable, with major loss of 8% of β -arbutin in one formulation having pH 3.3
- Both arbutins were found to be stable in aqueous/methanol solutions but unstable to strong hydrolytic conditions (2.5 h at 100 °C, pH 1.1)
- Both arbutins were rapidly metabolized in less than 8 h using pear peels as a surrogate *ex vivo* model.

Conclusions

- Both compounds presented similar stability profiles under selected chemical or biological conditions
- The stability in cosmetic formulations may be strongly dependent on the type of formulation and the pH
- Liquid formulations may be less stable than semi-solid preparations
- Both arbutins were stable in water or methanol up to 12 months with no generation of hydroquinone
- Both arbutins rapidly generate hydroquinone under strong hydrolytic conditions
- Both arbutin were unstable when exposed to enzymatic metabolism using pear peels as a source of β -glucosidases and peroxidases (known to regulate the accumulation of HQ/ARB *in vivo*)
- Hydroquinone was not found as a degradation product of arbutins after metabolic degradation
- Anomerization products were not found under any of the studied experimental conditions

Investigation of whitening agents in cosmetics

- **Aim:**

Investigation of the distribution of common whitening agents in cosmetic formulations
- **Method developed:**

Sample preparation for the simultaneous different types of skin whitening agents
HPLC-UV method for the simultaneous detection of 9 analytes
- **Experiments performed:**

HPLC-UV screening
- **Results:**

Fifty-nine skin whitening products including creams, lotions, sera, foams, gels, mask sheets, soap bars, tablets, and capsules were analyzed for the presence of common natural whitening agents, including α -arbutin, β -arbutin, kojic acid, nicotinamide, resorcinol, ascorbic acid, hydroquinone, and hydroquinone derivatives (4-methoxyphenol, and 4-ethoxyphenol)

Conclusions:

- The newly developed method enabled a baseline separation of nine analytes within 30 min.
- Arbutins (β - and/or α -), ascorbic acid, kojic acid, nicotinamide, hydroquinone, and resorcinol were identified in 27, 8, 8, 13, 10, and 3 products, respectively.
- No sample contained 4-methoxyphenol or 4-ethoxyphenol.
- From an overview of the 59 whitening products, more than 40% of the products were mislabeled.
- Thirty-four whitening cosmetic products claimed to contain either β -arbutin or α -arbutin. From these products, 50% contained β -arbutin, 29% contained α -arbutin, and 23% products did not contain either arbutin.
- One product contained 3.1% hydroquinone which was 163% of the listed amount on the product label. Hydroquinone in the range of 1.4 – 1.9% was found in four products. Three products were found to contain hydroquinone that was not listed on label.

CONCLUSIONS



- From a commercial point of view, arbutin is becoming a popular whitening agent positively accepted by the consumer as a “natural and safer alternative” to the golden standard, hydroquinone.
- Due to the economical interest in plant containing arbutin, a systematic analysis of plants known for their content of arbutin and related species has been performed. This survey enabled the identification of plants with high content of β -arbutin, which could potentially be added to cosmetic preparations as a natural source of arbutin
- From a toxicological point of view, it is vital to identify the potential risks of generation of hydroquinone upon storage and topical application. The performed stability studies confirmed the instability of arbutin in some cosmetic formulations (especially in liquid preparations and depending on the pH) after long term storage and upon enzymatic conversion. It is relevant to note that arbutin degradation did not necessarily correlate with an increase of hydroquinone content
- A survey of commercial preparations has also been performed in order to identify the most commonly used whitening agents which may possibly become objects of safety investigations in the near future

Comparative studies on the chemical and enzymatic stability of alpha and beta

arbutin

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Synopsis

OBJECTIVE: The aim of this study was to establish a comparative analysis of the chemical and enzymatic stability of α - and β -arbutins as potential source of the substance of concern hydroquinone. The study was performed using an array of techniques including HPLC-PDA, nuclear magnetic resonance and optical rotation. Both arbutins are emerging as popular and effective skin whiteners, by acting as tyrosinase inhibitors in a fashion similar to the popular whitening agent hydroquinone. Due to their structural similarity to the regulated agent hydroquinone, both arbutins may be regarded as potential sources of the active aglycone after chemical or metabolic conversion. **METHODS:** Various cosmetic formulations including creams, sera, gels and lotions were analyzed by HPLC-PDA for their arbutin and hydroquinone content in freshly opened and aged samples stored for 16 months. Solutions of pure compounds were also aged and periodically checked for degradation products by using 1D and 2D NMR experiments and optical rotation measurements. The metabolic stability was investigated using pear peels as a biological model. **RESULTS:** Both arbutins were found to be stable in water and methanol solutions in the absence of buffer or stabilizers. Their stability in cosmetic formulations however was found to depend on the type of formulation and pH. Both compounds were unstable under strong hydrolytic conditions, with consequent release of hydroquinone. Enzymatic instability of both arbutins was also observed, although no formation of hydroquinone was observed under the chosen experimental conditions. **CONCLUSION:** Both arbutins were found to possess similar stability profiles, and to be more prone to *in vivo* rather than *in chemico* degradation, although no hydroquinone was found after enzymatic hydrolysis. Also, no epimerization

was observed in any of the tested conditions. Diverse experimental approaches can be applied to analyze the chemical and enzymatic stability of arbutins in regard to the potential release of hydroquinone in different types of preparations. These result showed the potential use of NMR and OR as complementary investigative tools for the stability and safety assessment of arbutin along with more established HPLC methods.

Keywords

Chemical analysis, Formulation/stability, β -Arbutin, α -Arbutin, Hydroquinone, Whitening cosmetics

Introduction

Skin whitening agents are widely used in topical formulations for the treatment of pigmentation disorders, such as melasma, age spots or post-inflammatory hyperpigmentation, or for cosmetic purposes, to lighten or even the skin tone. In the last decade, the demand of skin-whitening products has increased steadily, becoming a multi-billion market, especially in countries where a lighter complexion is strongly associated to the idea of beauty. Among the several chemical agents, hydroquinone (HQ, **3**, Fig. 1S Supporting information) is one of the most prescribed ingredients. Hydroquinone inhibits the melanin synthesis by interfering with the enzymatic activity of tyrosinases, which convert the amino acid tyrosine into melanin [1]. Alpha- and beta-arbutins are glycoside derivatives of HQ, and also became popular skin whitening agents by acting in a similar fashion to their aglycone hydroquinone [2, 3]. β -Arbutin (4-hydroxyphenyl β -D-glucopyranoside, **1**, Fig. 1S) has achieved a positive acceptance among consumers, as a natural and effective alternative to hydroquinone and other toxic whitening agents [4, 5]. β -Arbutin occurs naturally in many plants of the *Ericaceae* and *Caprifoliaceae* families [6-8] and in pears (*Pyrus communis* L.) [9]. Unlike the β -anomer, α -arbutin (4-hydroxyphenyl α -D-glucopyranoside, **2**, Fig. 1S) is most commonly obtained by chemical or biotechnological routes [10].

Stability studies proposed so far mainly focused on the stability of β -arbutin, while less information is available for the α -anomer. Tong, *et al.* [11] assessed the stability of β -arbutin in a range of pH between 4 and 9, using a UV spectrophotometric method (not coupled to HPLC). Liu, *et al.* reported the stability of α -arbutin in different additives used in cosmetics

[12]. In any case, none of the existing methods provided a comparison between the two compounds. Analytical studies on cosmetic formulations are usually performed on creams [13]. Nonetheless, arbutin preparations on the market can be quite diverse in terms of physico-chemical features. Whitening cosmetic formulations range from creams to oil- or water-based gels and sera, to facial sheets, lotions and soaps. The final pH of the formulation and its specific composition, along with the packaging design, can dramatically affect the stability of arbutins by accelerating or slowing the aging process and the potential release of hydroquinone in the preparation. Formulations containing both arbutins can also be found on the market, thus methods to analyze and distinguish arbutins in complex formulations for quality control and stability studies are desirable. The development of traditional HPLC and UPLC methods can be challenging due to the close structural similarity between the two compounds, with elevated risk of co-elution, which can go unnoticed as both compounds possess identical mass and UV properties. Moreover, both arbutins can be regarded as a potential source of HQ, *in vivo* or after chemical decomposition. In the present work, a comparative study on the stability of α - and β -arbutin was undertaken using an array of diverse techniques to discriminate among the two structurally similar compounds. The effect of formulation and pH of commercial cosmetic preparations after 16 months storage was examined by HPLC-PDA. Stability studies have been also performed for both pure compounds under different chemical conditions using various analytical techniques, such as NMR, optical rotation and HPLC-PDA. Both arbutins may also generate hydroquinone *in vivo*, through skin metabolism or exogenous transformation by skin bacteria. For this reason,

enzymatic stability studies have been performed using pear peels as a biological model for *in vitro* studies.

Materials and Methods

Chemicals. α -Arbutin (**2**, CAS n. 84380-01-8) was acquired from Royal DSM (Heerlen, Netherlands). Hydroquinone (**3**, CAS n. 123-31-9) and β -arbutin (**1**, CAS n. 497-76-7) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The identity and purity was assessed using HR-ESI-MS, chromatographic methods and by ^1H and ^{13}C NMR. Pear samples were purchased from a local source (Oxford, MS 38655, USA). The pear variety used in this study was Red d'Anjou. Cosmetic products (including creams, sera, gels and lotions, C1-C9) containing α - or β -arbutin were purchased from the internet.

NMR studies. Four to 7 mg of pure compounds or mixtures was dissolved in 200 μL of deuterated solvent. ^1H and ^{13}C spectra of the samples were recorded to provide a reference standard. Three mm NMR tubes were used and the samples stored in the corresponding deuterated solvent at room temperature under artificial light for 8 h/day for 12 months. ^1H and ^{13}C spectra were periodically collected in the same conditions as for the standard compound. ^1H (500 MHz) and ^{13}C (126 MHz) NMR spectra were recorded on Agilent DD2-500 NMR spectrometers equipped with an oneNMR probe and Agilent Vnmrj 3.2 software. Chemical shifts were referenced to the residual solvent signal (CD_3OD : δ_{H} 3.31 ppm, δ_{C} 49.0 ppm, D_2O : δ_{H} 4.79 ppm). ^1H and ^{13}C -NMR experiments were performed at 25 $^\circ\text{C}$ (^1H parameters: resolution = 0.23 Hz, nt = 8, at = 5, d1 = 10 s, sw = 7485.0; ^{13}C parameters: nt=1000, d1= 3.5 s, sw = 31250.0). One-bond heteronuclear ^1H - ^{13}C correlation experiments were performed using gHSQCAD experiments (nt = 8, ni= 256).

Specific rotation. The samples to be analyzed for rotatory power were prepared in methanol or water (HPLC grade) according to literature data [14-16]. Solutions of α -arbutin were prepared to final concentrations of 2 g/100 mL in H₂O and 1.23 g/100 mL in MeOH; β -arbutin was diluted to final concentrations of 2 g/100 mL in H₂O and 0.56 g/100 mL in MeOH, respectively. The solutions were kept for a year under artificial light at room temperature for at least 8 h/day and specific rotatory power values were periodically acquired in the same standard conditions. A Rudolph Research Analytical digital polarimeter was used for the measure of the rotatory power. The readings were performed at 589 nm and 25 °C (corrected to 20 °C), using a 25 mm path length microcell.

HPLC-PDA analysis. The analytical investigations were performed on a Waters Alliance 2695 HPLC system using a data station with a Waters Empower 2 software. A Synergi Hydro RP column (250 \times 4.6 mm; 4 μ m) from Phenomenex was used, and the temperature was maintained at 45 °C. The column was equipped with a 2 cm LC-18 guard column (Phenomenex). The mobile phase consisted of water (A) and methanol (B) with both solvents containing 0.1% acetic acid. The analyses were performed according to a previous method [17]. The detection wavelength was 280 nm for both arbutins and hydroquinone.

Extraction solution and standard preparation for HPLC analysis. The extraction solution used for the sample preparation of standards, cosmetics and pear samples was obtained by mixing 10% MeOH in 20 mM NaH₂PO₄ buffer (v/v, pH 2.3). An individual stock solution of standard compounds **1-3** were prepared at a concentration of 5.0 mg/mL in the extraction solvent.

Chemical hydrolysis. 0.2 Milligram of β -arbutin or α -arbutin was accurately weighed and

dissolved individually in 1.0 mL 10% MeOH in acid condition (pH 1.1). The sample was heated in boiling water for 2.5 h, then analyzed by HPLC-PDA.

Investigation of skin whitening formulations. The cosmetics used were creams, sera, lotions and gels. The pH of the various cosmetic preparation was measured according to the European guidelines [18]. A 10% solution or dispersion of the product was prepared by dissolving 1 g of cream in 10 g of HPLC grade water. The pH values were measured in triplicates and the results were averaged. A pH meter (Model IQ125 Professional, IQ Scientific Instrument, Inc., Carlsbad, CA, USA) was used and calibrated with reference solutions pH 4.0, 7.0 and 10.0 before the measurements.

The skin whitening samples were prepared for HPLC analysis as follows. Five hundred milligram of each product was accurately weighted into a 15 mL centrifuge tube and 8 mL of the extraction solution was added. The samples were vortexed and sonicated for 30 minutes. After sonication, the samples were centrifuged at 4000 rpm for 30 min and the supernatant was transferred into a 10 mL volumetric flask. Two mL of the extraction solution was added into the centrifuge tube and sonicated again for 30 min. After centrifugation, the supernatants were combined, and the volume was adjusted to 10 mL with the extraction solution. The extracts were mixed thoroughly, and filtered with 0.45 μ m PTFE filter prior to HPLC analysis.

Spiking and extraction of pear samples. The epicarp of 12 fresh pears was ground after separation from the pulp. Two grams of the ground sample was accurately weighed into a 15 mL centrifuge tube and extracted by sonication using 3 mL of extraction solvent. The samples were centrifuged at 4000 rpm for 15 min and the supernatant was transferred into a

10 mL volumetric flask and the procedure repeated 2 more times. After centrifugation, the supernatants were combined, and the volume was adjusted to 10 mL with the extraction solution. The extracts were mixed thoroughly, and filtered with 0.45 μ m PTFE filter prior to HPLC analysis. Spiked samples were obtained by adding 100 μ L of a solution of either α - or β -arbutin (10 mg/mL H₂O).

The spiked samples were incubated at room temperature for 0, ½, 1, 2, 4 and 8 h respectively. After incubation, the enzymatic activity was quenched by addition of extraction solution (3 mL) and extracted as explained for the fresh pears samples.

Control samples were prepared following the procedures by pre-treating the plant material to deactivate the enzymes, removing the β -arbutin present in the fresh peels, and spiking a known amount of β - or α -arbutin standard compounds. Forty grams of fresh ground peels were accurately weighed and split in four 50 mL centrifuge tubes. The samples were extracted with methanol (45 mL) and centrifuged at 4000 rpm for 30 min. The procedure was repeated four times then the sample were extracted with water (50 mL) and centrifuged for two more times to remove the excess of methanol. The remaining solid material was weighed and adjusted with water to restore the initial amount of water content. Two grams of the obtained sample was then accurately weighed in a 15 mL centrifuge tube, the samples were spiked with β - or α -arbutin and extracted as explained above.

Results and Discussion.

Stability of arbutins in cosmetic formulations.

In order to investigate the effect of pH and formulation on the stability of **1** and **2** in cosmetic preparations, nine types of products were analyzed. Two samples were found to have pH values above 8 and one less than 4 (Table I). The samples were all stored at room temperature for 16 months to mimic the typical storage conditions used by the consumer, then re-analyzed by HPLC-PDA under the same conditions as the freshly opened samples. Five samples out of 9 were found to contain above 95% of the initially determined α - or β -arbutin content. All the sera samples (C3, C5, C9) showed losses of arbutin content between 5.8 and 20.7%. Recovery within experimental error was found for creams, lotions and gel formulations, with the exception of sample C2, which was also determined to have a pH value of 3.3. As summarized in Table I, pH values above 4 didn't affect the stability of either arbutin in non-liquid preparations (C1, C4, C6, C7, and C8) under the tested conditions, even after 16 months. On the contrary, the nature of the formulation may affect the stability of compounds **1** and **2**. Both arbutins were found to be less stable in liquid preparations, with a major loss in the presence of basic solutions, such as sample C5. β -Arbutin is reported to be a highly photosensitive compound [19], and sunscreen agents are often added in whitening cosmetics to prevent β -arbutin degradation. After UV exposure, the pH conditions may affect the stability of the compounds, and accelerate the decomposition rate. Several stability studies have been reported in the literature for β -arbutin, with differing outcomes [20, 21]. Our results confirmed that both arbutins may be unstable with dependence on the pH and

type of formulations, thus both factor have to be accounted for in the design of new formulations.

Chemical stability studies.

Based on the results obtained from long term storage of different whitening formulations, additional studies on the stability of pure compounds in water or alcoholic (methanol) solutions were performed in the absence of acid or bases to verify if the instability of arbutin may have been related to a photo-catalyzed degradation independent from the pH conditions. These studies have been performed using Nuclear Magnetic Resonance (NMR) or optical rotation (OR) analysis. The samples were stored in NMR tubes or clear scintillation vials at room temperature under normal light irradiation for at least 8 h/day. Although less sensitive than HPLC, NMR can provide very useful molecular insights, especially when comparing structurally similar compounds, such as α - and β -arbutin, containing identical functional groups but different structural orientation. The NMR spectra of **1** and **2** possess unique ^1H and ^{13}C features at the anomeric position. The anomeric proton signal of β -arbutin at 500 MHz can be identified as a doublet at δ 4.74 in CD_3OD (Fig. 2Sa, Supporting Information), or at δ 4.96 in D_2O (Fig. 3Sa), while for α -arbutin, the same signal can be observed at δ 5.29 in CD_3OD or δ 5.46 in D_2O , respectively (Figs. 2Sd and 3Sd). The coupling constants for the anomeric proton were found to be $J = 7.3$ Hz for the β - and $J = 3.7$ Hz for the α -anomer. These values are in agreement with a predicted diaxial coupling ($J = 7\text{--}9$ Hz) and axial-equatorial coupling ($J = 2\text{--}4$ Hz) which are associated to a β - and α -configuration, respectively [22]. The ^{13}C NMR spectra of the two compounds are also very similar, with

the exception of the C1' position, which is also affected by the sugar configuration [23]. The observed chemical shifts at 500 MHz were 103.2 ppm and 103.9 ppm (D₂O, Fig. 5Sa) in the case of β -arbutin and δ 100.2 (CD₃OD, Fig. 4Sd) and δ 100.4 (in D₂O, Fig. 5Sd) for α -arbutin. The samples of pure α - or β -arbutin were stored in NMR tubes and analyzed at different time intervals (see Figs. 2-5S). If hydrolysis resulting in the generation of hydroquinone and D-glucose occurred, the presence of a new ¹³C signal at 96 ppm would be expected. No signals at 96 ppm were detected for either arbutin, thus confirming the lack of hydrolysis events in all the analyzed samples, even after 12 months. In order to confirm the finding from 1D ¹H experiments, all the samples were also analyzed using 2D hetero-correlation analysis, where the anomeric signals can be clearly identified without residual solvent interference. The results from gHSQCAD analysis are presented in Fig. 6S and 7S. The 2D NMR experiments confirmed the absence of release of free glucose or anomerization processes in the analyzed samples.

In a similar fashion to the NMR study, sample solutions were analyzed for changes of the rotatory power in non-deuterated methanol and water at the same conditions reported in the literature [15, 16, 24] (Table IS, Supporting Information). The measured OR values were stable within the precision of the instrument for six months. The results from both NMR and optical rotation experiments with solutions of pure standard compounds supported the statement from the opinion of the European Commission on β -arbutin, which reports the compound as “stable under sunlight when kept at 3% solution in ethanol in transparent glass bottle for 30 days” [25]. Similar results were also obtained for α -arbutin. Minor degradation peaks (below the LOQ of the detection method) in ¹H NMR spectra were observed after 12

months, but none of these signals have been correlated to the formation of glucose as a result of hydrolysis. Also, no epimerization was observed under any of the experimental conditions tested. Both NMR and optical rotation results didn't indicate the presence hydroquinone as a degradation by-product.

To verify that hydroquinone can be chemically obtained as degradation product of arbutins, a standard chemical hydrolysis procedure [26] was also performed and the HPLC results are shown in Fig. 1. Both arbutins were found to be readily hydrolyzed in 2.5 h under strong acidic (pH 1.1) and temperature conditions, with a complete loss of arbutin and generation of hydroquinone as major degradation product. The yields of hydroquinone converted from β - and α -arbutin were 84.9 and 89.6%, respectively.

Enzymatic stability studies.

The accumulation of β -arbutin in pear tissues is enzymatically controlled [27, 28], an observation which has been related to the potential role of β -arbutin in the defense mechanism from pathogens like fireblight [29-33]. Pear peels are known for their content of β -glucosidases and peroxidases as major enzymes involved in the arbutin metabolism [29, 34]. Peroxidases are also involved in melanogenesis and human skin metabolism [35]. The fates of both **1** and **2** *in vivo* were thus determined using pear peels as surrogate *in vivo* models. The concentrations of both arbutins and hydroquinone were evaluated by HPLC-PDA. The initial content of β -arbutin present in pear peels was estimated to be 0.138 mg/g in freshly prepared samples. The concentration of β -arbutin rapidly decreased in less than 8 h, with a total loss of > 90% after 24 h (Fig. 2a). The peel samples were then spiked with

269 either β - (Fig. 2b) or α -arbutin 0.5 mg/g (Fig. 2c). After 8 h, a loss of 62.4 and 49.4% of α -
270 and β -arbutin, respectively, were measured. In the control samples, which were pre-treated
271 in MeOH to quench the enzymatic activity, the loss of arbutins was negligible even after 24
272 h, thus excluding the possible role of external factors (e.g. light, air exposure, heat) in the
273 experimentally observed degradation processes. From the PDA UV profile (Fig. 8S,
274 Supporting Information), no hydroquinone was generated during the decomposition of either
275 arbutin. Any peak at the retention time of hydroquinone (12.57 min) was below the limit of
276 detection even after 24 h. β -Arbutin is known to hydrolyze enzymatically to hydroquinone,
277 and some of the biological and toxicological properties of β -arbutin can be directly related
278 to its aglycone after *in vivo* metabolism [36, 37]. Bang *et al.* reported the potential release of
279 hydroquinone *in vivo* through enzymatic hydrolysis of β -arbutin by skin microflora [38].
280 However, the results herein presented demonstrated that arbutin metabolism may not
281 generate hydroquinone as unique by-product. The authors hypothesize that hydroquinone
282 may be formed after enzymatic hydrolysis but not detectable due to the chemical or
283 enzymatic instability of the compound in the chosen experimental conditions. Short term
284 studies on solutions of hydroquinone at different pH conditions demonstrated that the
285 compound partially degrades at pH>5, with a loss of 20% of hydroquinone at pH 7.6 after
286 24 h (data not shown), thus the compound may be generated *in vivo* and rapidly degrade due
287 to the chemical conditions of the cellular environment. Another hypothesis is derived from
288 the observation that both hydroquinone and arbutin are potential substrate of peroxidases
289 [34, 39]. It is thus possible that either hydroquinone is not released in *in vivo* conditions or

it is rapidly converted into more stable end-products. Further studies will be necessary to confirm whether the observed results using pear peels can be translated to skin metabolism.

Conclusions

The long term stability of arbutins in cosmetic formulations stored for 16 months was found variable depending on the formulation and pH, with a partial loss in basic liquid preparations. Both arbutins were found to be stable in neutral solutions (in the absence of stabilizers or buffers). On the other hand, both **1** and **2** were found to be unstable under strong hydrolytic conditions with consequent formation of hydroquinone. Metabolic instability of both arbutins also need to be considered as a potential concern, although the potential release or accumulation of hydroquinone in the skin after topical application of arbutins may not necessarily occur.

303

304 **Acknowledgments**

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310

311 **Conflicts of Interest**

312 The authors declare no conflict of interest.

313

314

315 **References**

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Figure Legends

Fig. 1 HPLC-PDA chromatograms of β -arbutin, α -arbutin, and related products after hydrolysis (**1**, β -arbutin; **2**, α -arbutin; **3**, hydroquinone)

Fig. 2 Stability studies results using fresh pear peels. (—) Control (- - -) reaction A) degradation kinetic of β -arbutin naturally occurring in the fresh peels, B) Sample spiked with 0.5 mg/g of β -arbutin, C) degradation kinetic of α -arbutin in samples spiked with 0.5 mg/g compound.

Table I. Stability study on commercial whitening formulations.

			β -Arbutin			α -Arbutin		
CODE	Formulation	pH	In. amount (mg/100 mg)	16 month (mg/100 mg)	Recover (%)	In. amount (mg/100 mg)	16 month (mg/100 mg)	Recover (%)
C1	cream	8.5	0	0	-	1.68	1.76	105.3
C2	cream	3.3	3.20	2.95	92.2	0.00	0.00	-
C3	serum	7	0.00	0.00	-	2.10	1.94	92.4
C4	cream	7.6	0.00	0.00	-	0.15	0.16	106.7
C5	serum	8.8	0.21	0.17	79.3	0.00	0.00	-
C6	gel	4.4	0.13	0.13	100.0	0.00	0.00	-
C7	cream	7.7	0.00	0.00	-	1.01	1.03	102.0
C8	lotion	6.7	3.10	3.05	98.5	0	0	-
C9	serum	4.3	4.30	4.05	94.2	0	0	-

Table I.

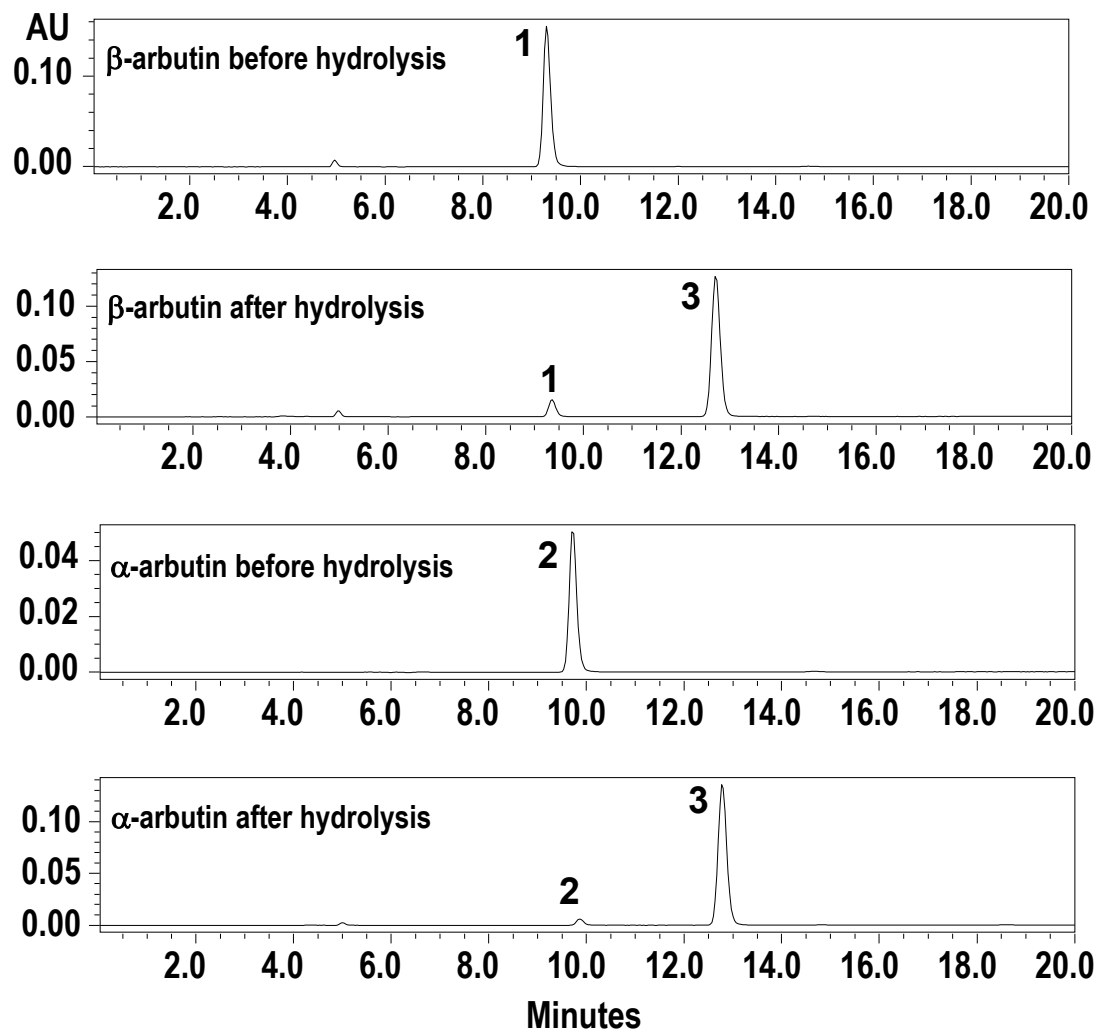


Figure 1

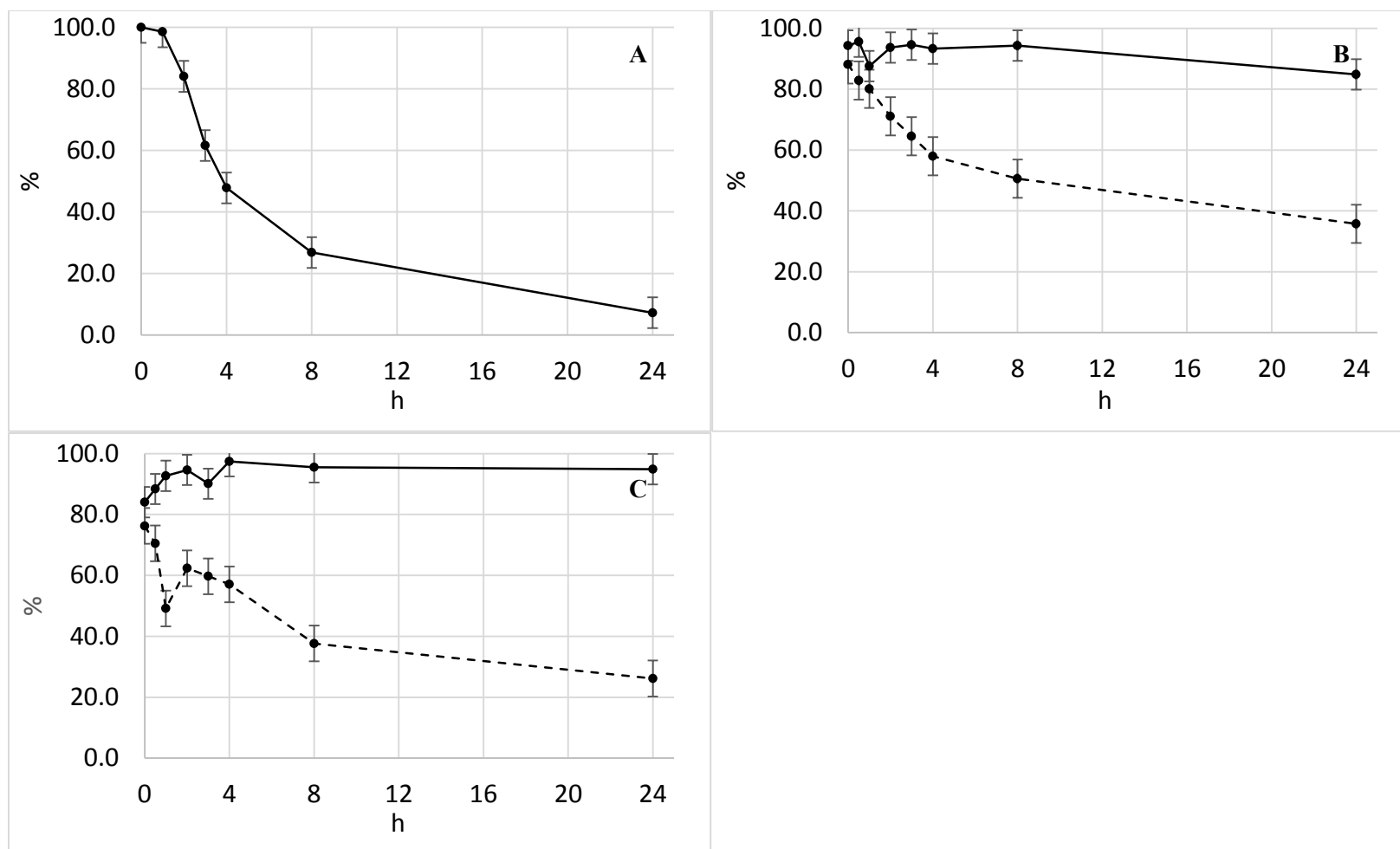


Figure 2

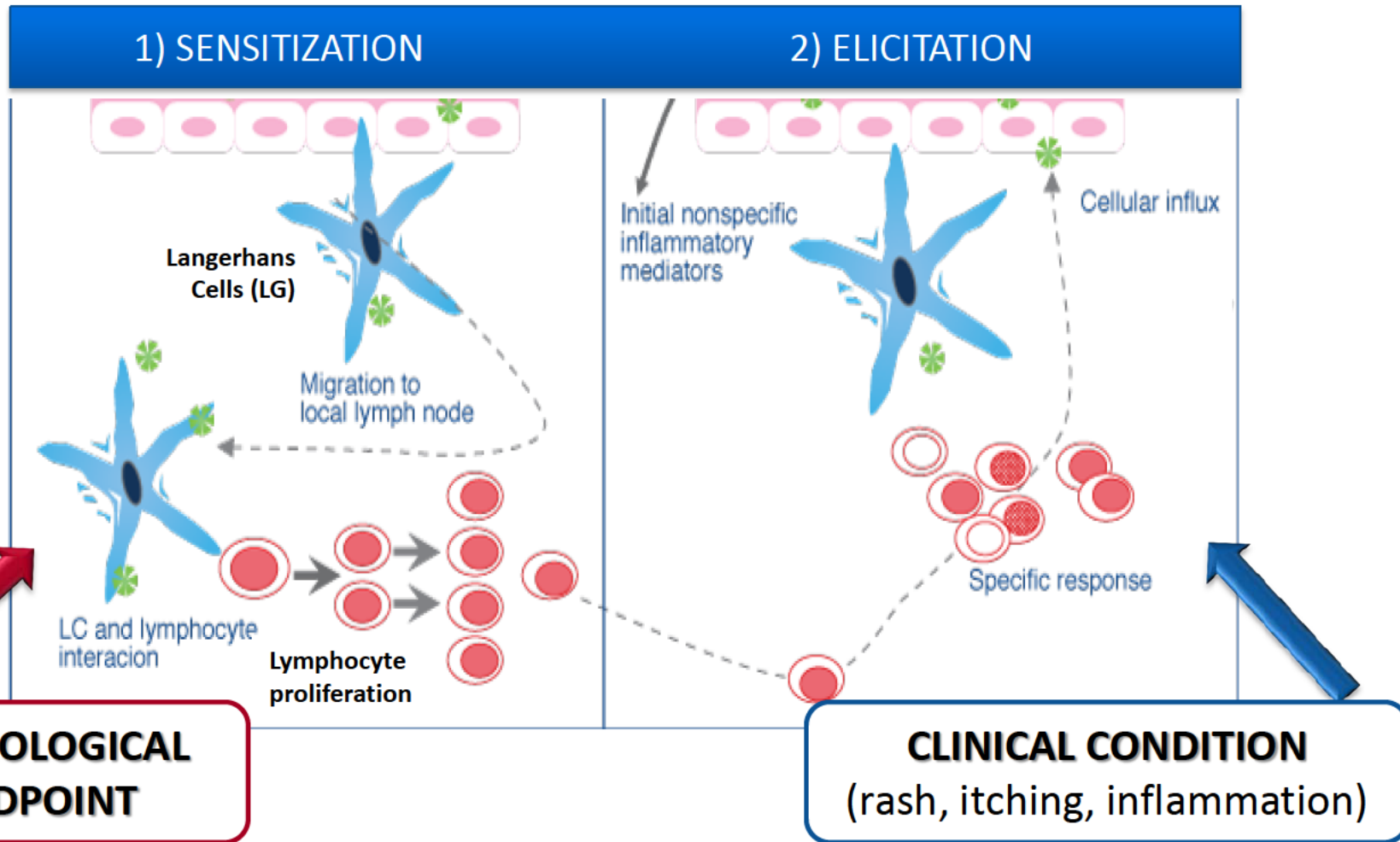
In chemico screening for skin sensitization risk assessment

**15th Annual Oxford International Conference
on the Science of Botanicals**
Monday, April 13th 2015

Cristina Avonto, Ph. D.
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SKIN SENSITIZATION

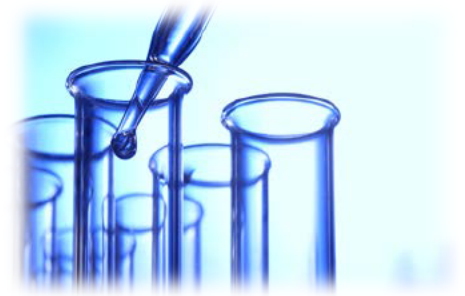
Skin sensitization refers to the first phase of a form of chemical allergy known as Allergic Contact Dermatitis (ACD)



AN INTEGRATED APPROACH

- The replacement of animal tests for risk assessment has become a major priority for regulatory agencies worldwide
- No individual *ex vivo* assay is suitable as a stand-alone alternative to animal tests
- An integrated approach will allow to combine results from chemical, biological and computational data in one final assessment of the Adverse Outcome pathway (AOP)

In chemico



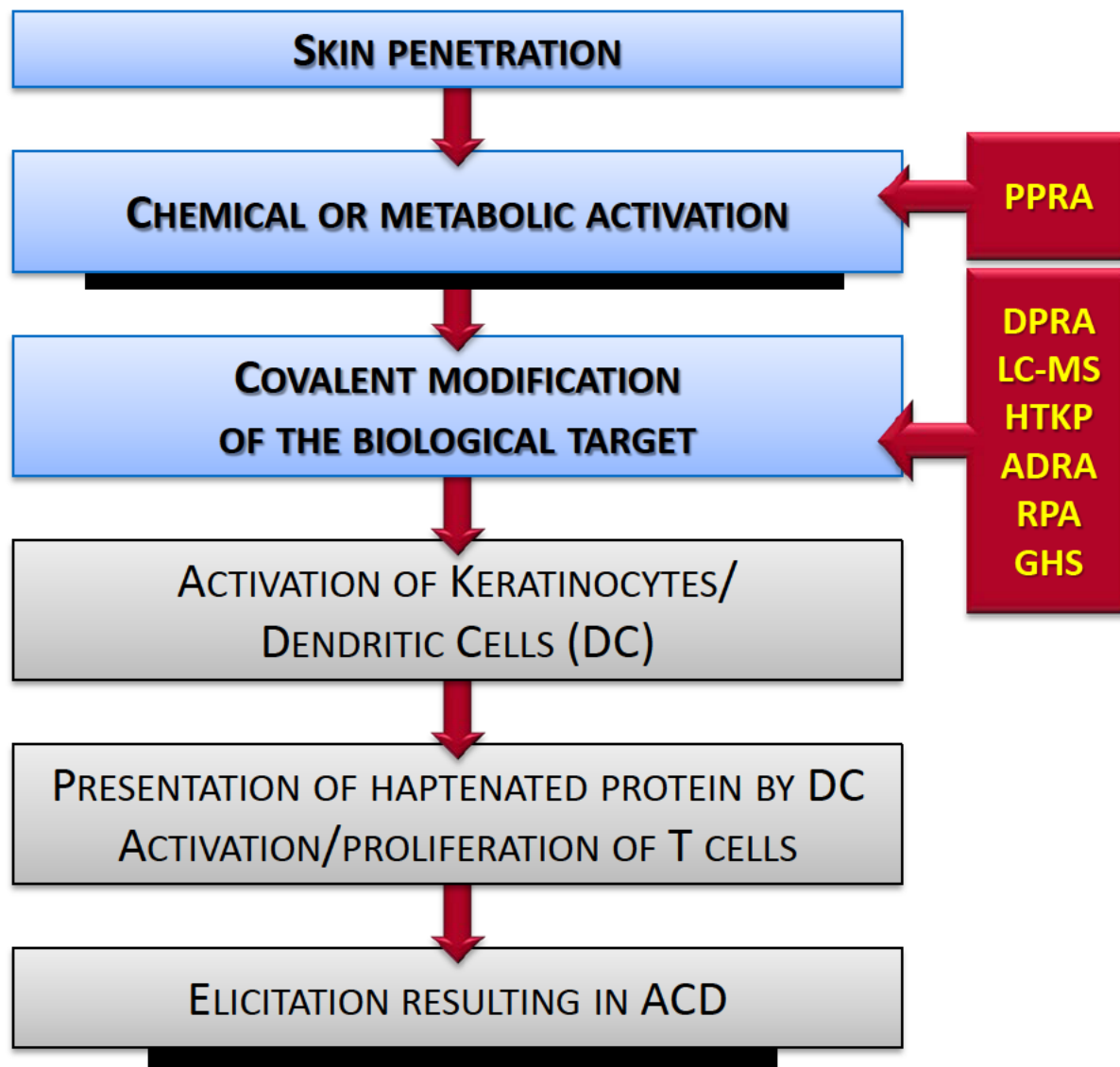
In vitro



In silico

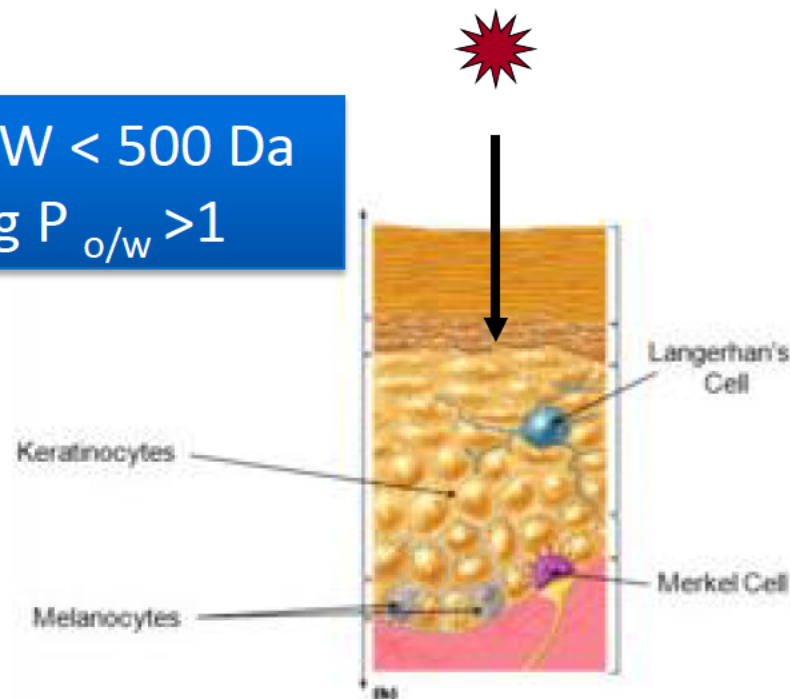


AN INTEGRATED APPROACH



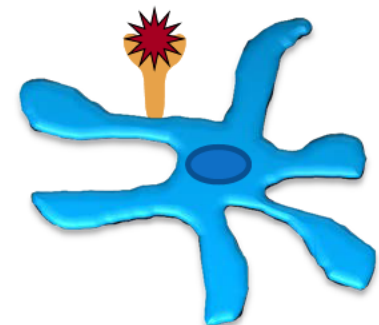
THE HAPTEN PARADIGM

- In order to act as a sensitizer, a chemical compound has to penetrate the lipophilic *stratum corneum* which acts as a primary defense against chemical, mechanical and biological hazards
- Once in the epidermis, the chemical has to covalently bind to the biological target to induce a specific T lymphocyte response



1. MW < 500 Da
2. $\log P_{o/w} > 1$

3. CHEMICALLY REACTIVE



MECHANISTIC DOMAINS IN ACD



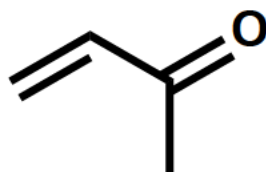
Nucleophile
(Cys, Lys)



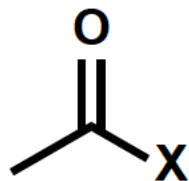
Electrophile



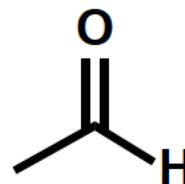
S_N1/S_N2
electrophiles



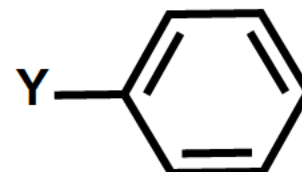
Michael
acceptors



Acylating
agents



Schiff base
initiators



S_NAr
electrophiles

X= electron withdrawing group
Y= halogen/pseudohalogen

AN INTEGRATED APPROACH

Recently approved non-animal methods¹:

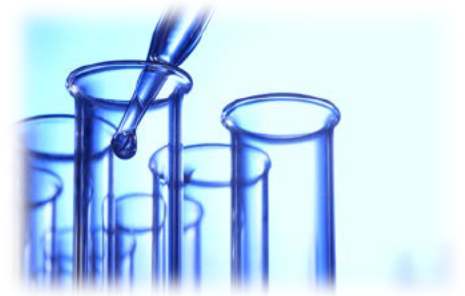
In chemico

- ✓ Direct Peptide Reactivity Assay (DPRA)

In vitro

- ✓ Keratinosens™
- ✓ Lusens
- ✓ MUSST (Myeloid U937 Skin Sensitization Test)
- ✓ hCLAT (human Cell Line Activation Test)

In chemico



In vitro



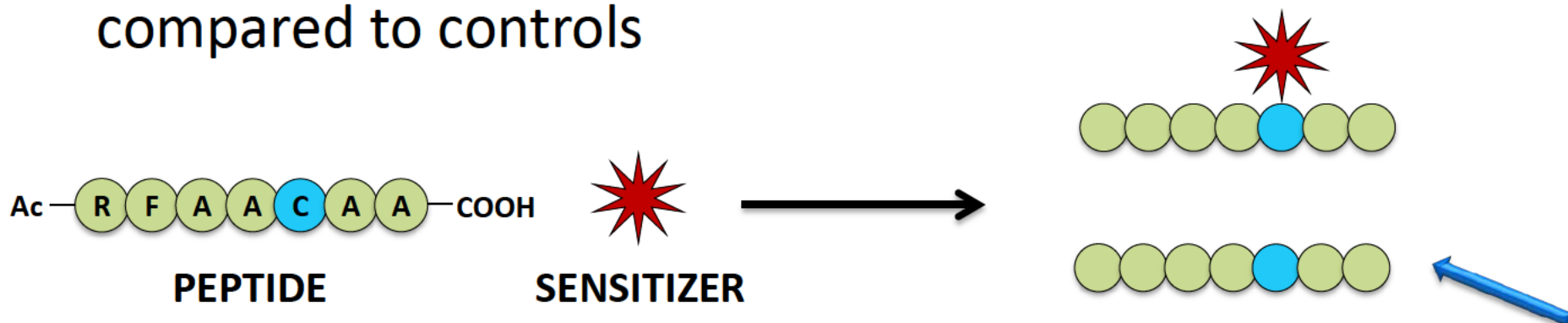
In silico



¹ Urbisch, D. et al. *Regul Toxicol Pharmacol* (2015), 71, 337-351

DIRECT PEPTIDE REACTIVITY ASSAY

- Model peptide containing Cys, Lys or Hys as reactive nucleophilic amino acid¹
- The peptide (in aqueous buffer) is mixed with an excess of candidate sensitizer (in ACN or DMSO) and incubated for 24 h at RT prior to HPLC analysis
- The percent of *peptide depletion* is determined as the reduction of the unmodified peptide concentration compared to controls



¹ Gerberick, G. *et al. Toxicol Sci* (2004), 81, 332-343

OTHER *IN CHEMICO* METHODS (1)

- LC-MS Peptide Reactivity Assay¹
- High Throughput Kinetic Profiling assay²
- Peptide-Peroxidase Assay³

¹ Natsch, A. *et al. Toxicol sci* (2008), 106, 464-478

² Roberts, D.W. *et al. Chem res toxicol* (2009), 22, 592-603

³ Gerberick, G. *et al. Toxicol Sci* (2009), 112, 164-174

OTHER *IN CHEMICO* METHODS (2)

- Amino acid Derivative Reactivity Assay¹
- Reactivity Profiling assay²
- Micro-emulsion systems³
- Kinetic assays using glutathione^{4,5}
- UV-Stopped-flow techniques using nitrobenzene thiol⁶

¹Fujita, M. *et al. J Pharmacol Toxicol Methods* (2014), 70, 94-105 ²Aleksic, M. *et al. Toxicol Sci* (2009), 108, 401-411

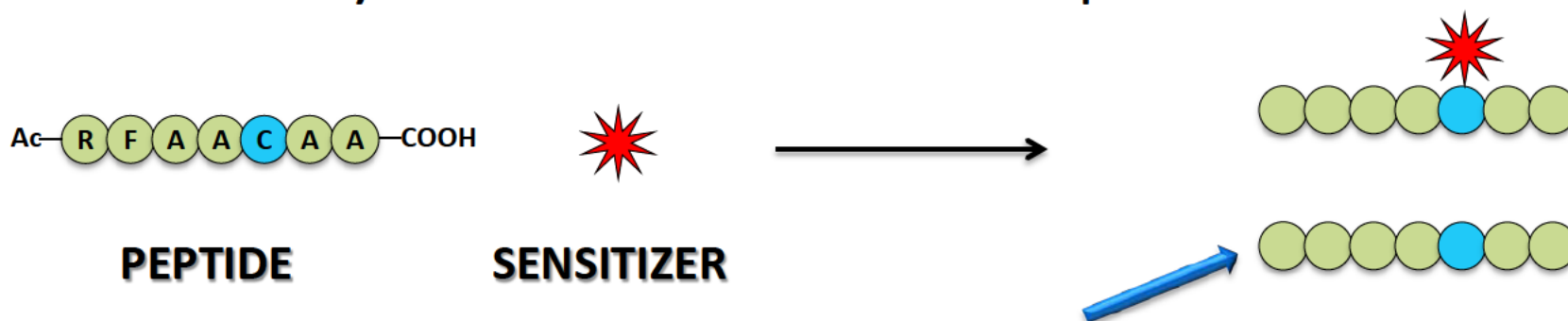
³Merckel, F. *et al. Chem Res Toxicol* (2010), 23, 1433-1441

⁴Schultz, T. W. *et al. SAR QSAR in Environ Res* (2005), 16, 313-322 ⁵Böhme, A. *Chem Res Toxicol* (2009), 22, 742-750

⁶Chipinda, I. *et al. Chem res toxicol* (2010), 23, 918-925

LIMITS OF EXISTING METHODS

- Indirect detection of the reaction adducts
- Drowning-out effect
- No information on the mechanistic domain or adducts characterization
- Validated only with pure compounds
- Low throughput
- Time delay between first and last sample



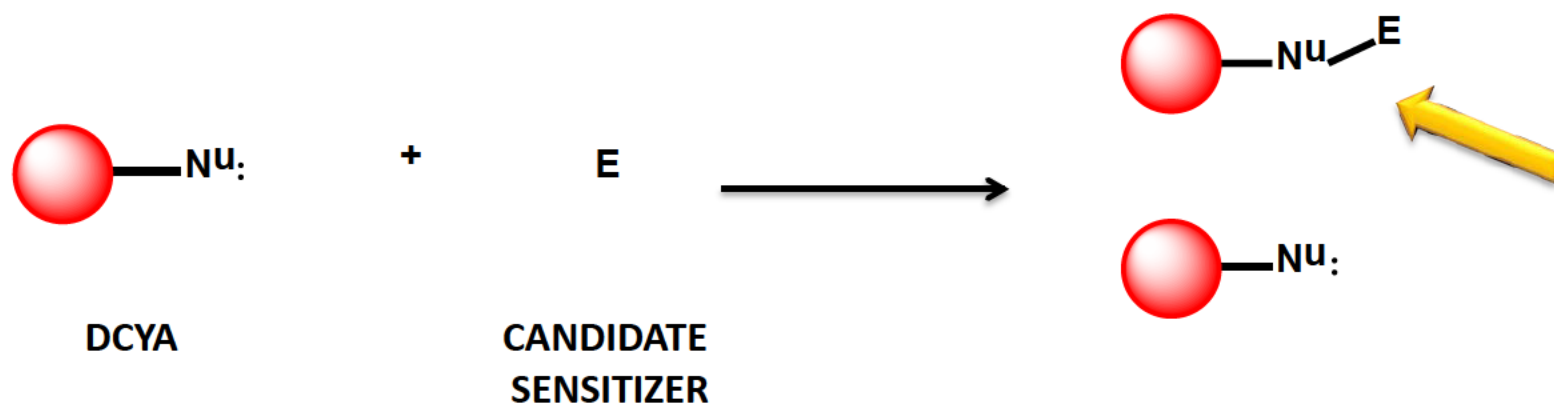
THE DCYA ASSAY



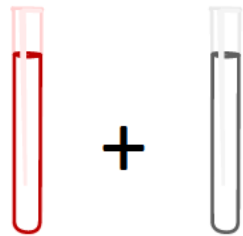
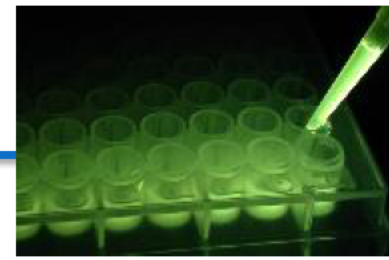
THE DCYA ASSAY



The proposed method is based on a designed lipophilic *fluorescent nucleophile* as a “chemical trap” for electrophilic compounds



THE DCYA ASSAY

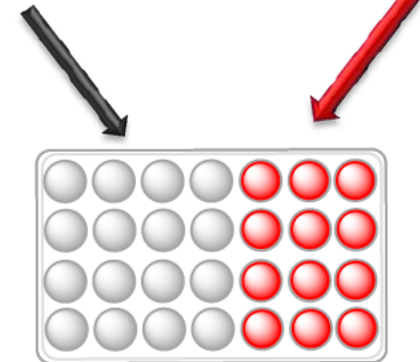


1. DCYA ACTIVATION
2. REMOVAL OF UNREACTED DCYA



NON
SENSITIZER

POTENTIAL
SENSITIZER



DCYA IS MIXED WITH
THE POTENTIAL
SENSITIZERS

QUANTIFICATION OF THE
FLUORESCENCE RESPONSE

METHOD VALIDATION



SAMPLE	COMPOSITION	RESULT
Positive Control (PC)	DCYA	Maximum expected reading
Negative Control (NC)	DCYA+ Activator	Background response
Blank (Bl)	DCYA + Sensitizer	“electrophile” interference
Reaction (R)	DCYA + Sensitizer + Activator	The sample reactivity

$$\text{Reactive Index (RI)} = 100 \left\{ \frac{Bl - R}{PC - NC} - \frac{PC - Bl}{PC} \right\}$$

METHOD VALIDATION



SAMPLE	SENSITIZATION CLASSIFICATION ¹	DCYA (100-RI)	DPRA ¹ (% pept remaining)	Keratinosens ¹ EC3 (μM)	LLNA ¹ EC3 (%)
2-Methyl-4-isothiazolin-3-one	STRONG	16.4	2.1	29.56	1.9
Cinnamaldehyde	MODERATE	85.1	29.4	63.94	3.0
<i>t</i> -2-Hexenal	MODERATE	29.9	2.1	374.57	5.5
Farnesal	WEAK	94.8	83.6	>2000	12
Benzyl benzoate	WEAK	110.6	99.8	142.47	17
Cinnamyl alcohol	NON REACTIVE	105.0	100	>2000	21
Coumarin	NON REACTIVE	97.2	99.0	479.96	NC



¹Natsch, A. *et al. J Appl Toxicol* (2013), 33, 1337–1352

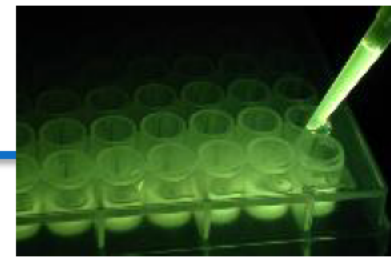
THE DCYA ASSAY



ADVANTAGES

- Ideal for a quick and cheap screening of large chemical libraries
- Results comparable with DPRA
- Mixtures and plant extracts can be analyzed
- Minimization of side reactions and false positives
- Improved solubility
- Minimum amount of reagents required
- Compatible with other analytical methods

THE DCYA ASSAY

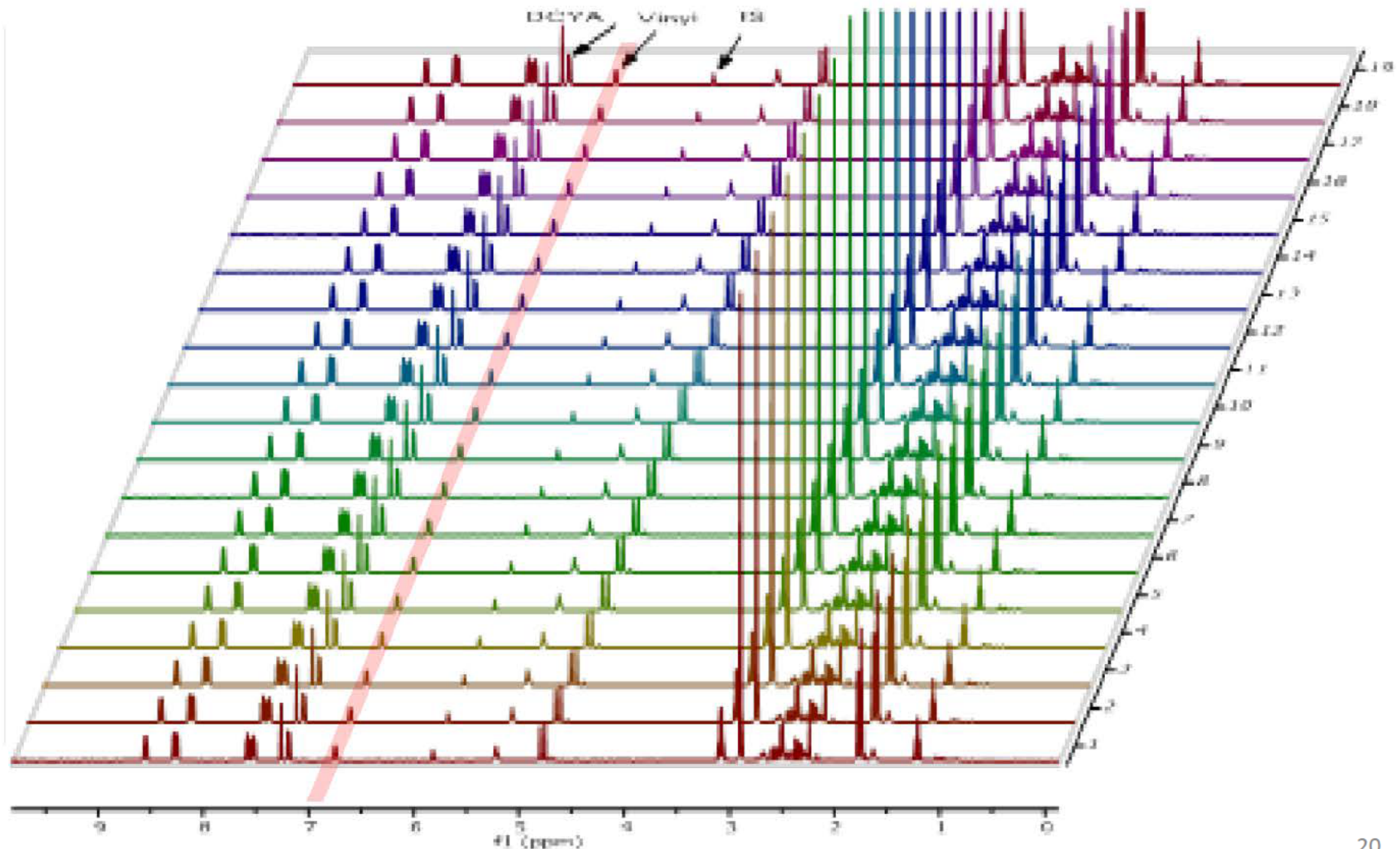


LIMITATIONS:

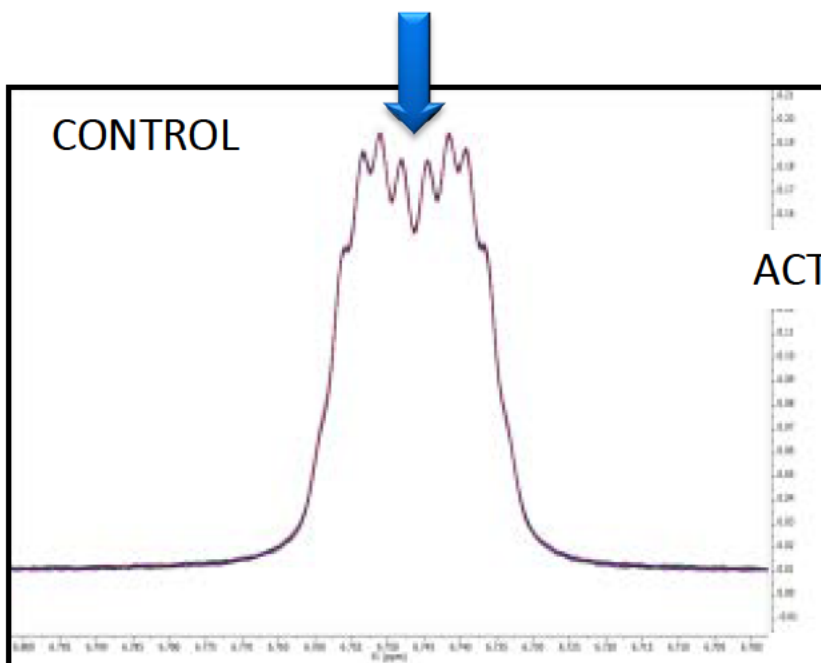
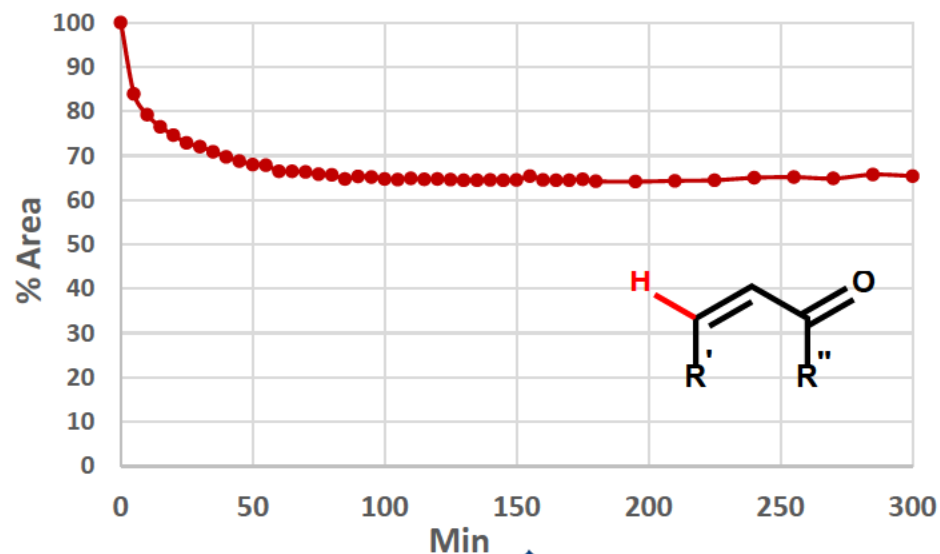
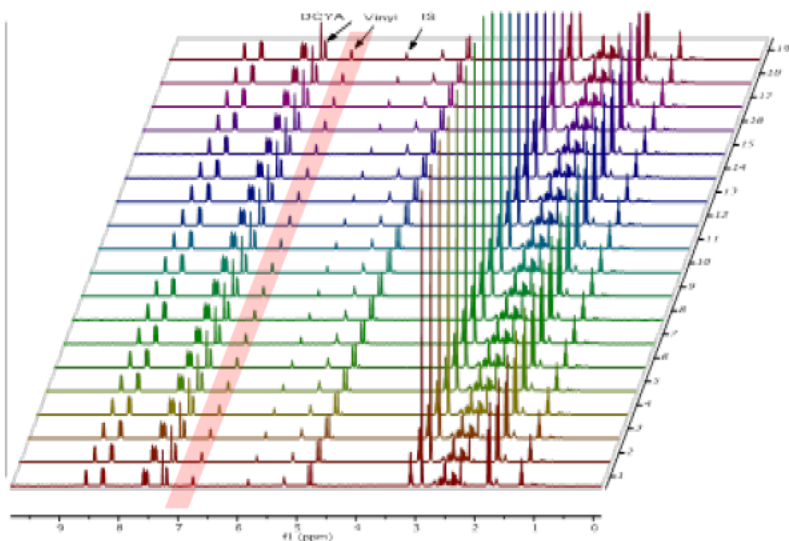
- Pre/pro-haptens cannot be detected
- Solubility issues with highly polar compounds
- The reaction time is critical for the quantification
- *No insight on the mechanism of the reaction*

**COMPLEMENTING
IN CHEMICO SCREENINGS
WITH STRUCTURAL ANALYSIS:
THE kNMR ASSAY**

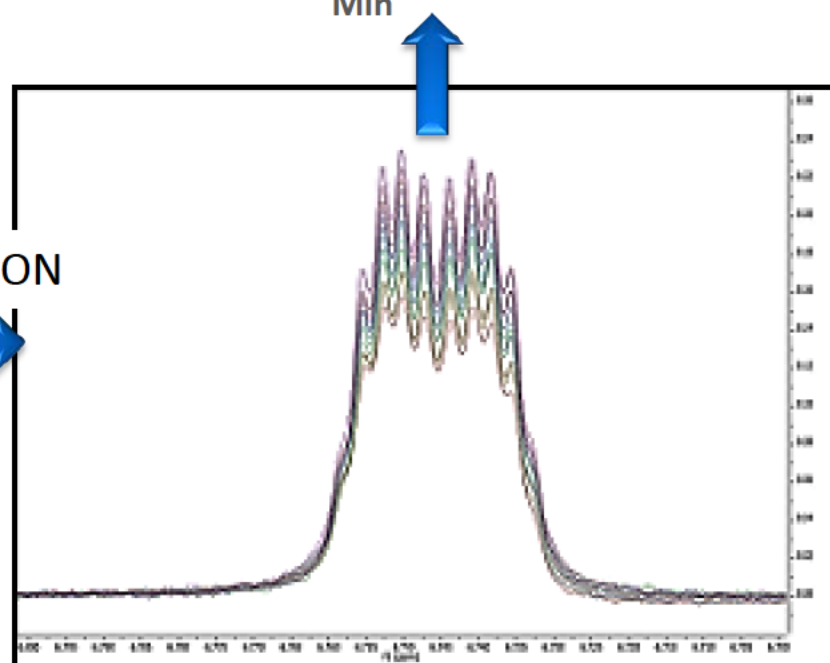
kNMR DCYA ASSAY



kNMR DCYA ASSAY



ACTIVATION



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- Ikhlas A. Khan
- Amar Chittiboyina
- Diego Rua, FDA
- Yelkaira Vasquez
- Mei Wang
- Jon F. Parcher

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TOOLS FOR ESTIMATION OF SKIN SENSITIZATION POTENTIAL

METHODS AVAILABLE AT UM

EX VIVO ASSAYS AVAILABLE AT UM

IN CHEMICO

Spectrophotometric High Throughput Screening method (HTS-DCYA)

Nuclear Magnetic Resonance (NMR) spectroscopic method (NMR-DCYA)

IN VITRO

Human Cell Line Activation Test (h-CLAT)

HTS-DCYA ASSAY

Fluorescent spectrophotometric method recently developed at UM¹

Features

The developed method is ideal for the *simultaneous* analysis of multiple test articles

Mixtures of multiple components can also be *qualitatively* analyzed for the content of reactive components

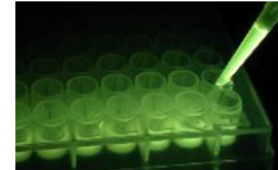
Advantages

- ✧ Minimum sample requirements
- ✧ Comparable to methods approved by regulatory agencies (Cys-DPRA)
- ✧ Ideal for pre-screening of mixtures for Substances of Concern (SoC)

Limitations

- Pre- or pro- sensitizers may require chemical/metabolic activation before the testing

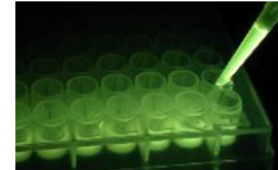
¹U.S. Provisional Application Serial No. 62/017,586; Manuscript under review, TAAP



HTS-DCYA ASSAY

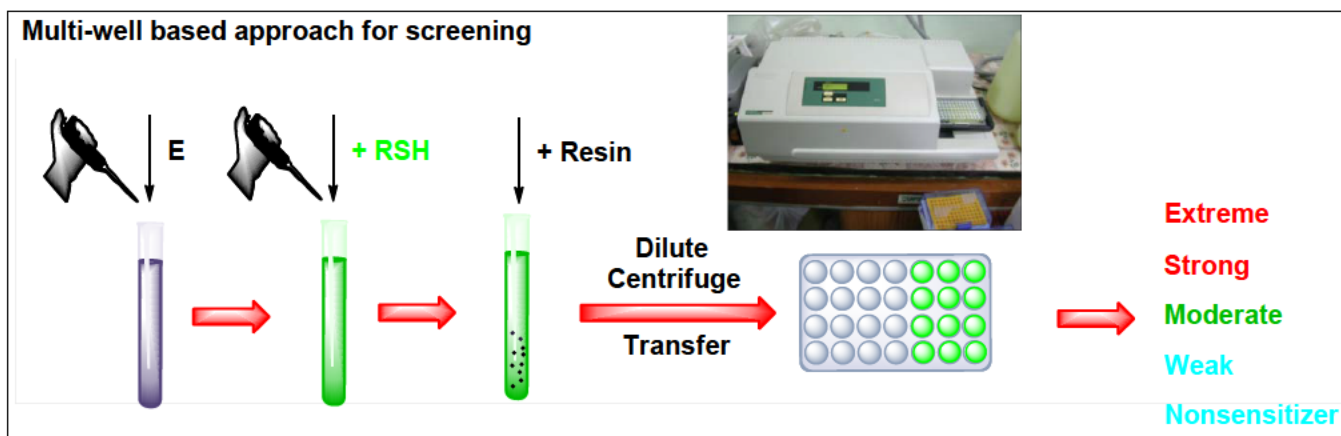
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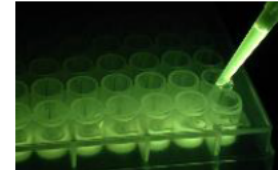
- *In chemico* method based on the reaction of the candidate sensitizer (test article) with a model fluorescent nucleophile (DCYA)
- After incubation, the unreacted nucleophile is removed and the fluorescent adduct, DCYA-sensitizer, is quantified
- The fluorescence response is directly proportional to the reactivity of the potential sensitizer; i.e., strongly reactive sensitizers result high fluorescence response, whereas non-sensitizers would result no-to-minimal fluorescence response.



HTS-DCYA ASSAY

Flow diagram of spectrophotometric method





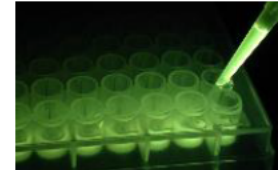
HTS-DCYA ASSAY

Method validation

- A total of 33 compounds* were chosen for the validation of method (HTS-DCYA) described in the previous slides
- Pre-/pro-haptens, non-sensitizers, weak, moderate and strong/extreme sensitizers have been included
- Based on Reaction Indices, further binary analysis was applied for comparison with approved *in chemico* methods (*viz.*, Cys-DPRA)**

*The list of compounds used for the method validation included Diphenyl cyclopropenone, *p*-Benzoquinone, 1-Chloro-2,4-dinitrobenzene, *p*-Hydroquinone, Propionolactone, 3-Hydroxytyrosol, 1,2-cyclohexanedicarboxylic anhydride, 2-Methyl-4-isothiazolin-3-one, Cinnamaldehyde, 2,4-Heptadienal, 4-Hex-3-en-one, Squaric acid, *t*-2-Hexenal, Resorcinol, Diethyl maleate, Safranal, Perillaldehyde, Citral, Farnesal, L-Carvone, Oxalic acid, Benzyl Benzoate, Lilial, Cinnamyl alcohol, *cis*-6-Nonenal, 5-Methyl-2,3-hexanedione, Ethyl acrylate, Aniline, 1-Bromobutane, Vanillin, Tartaric acid, Chlorobenzene, Lactic acid, Salicylic acid, Coumarin, Benzaldehyde

**Statistical analysis excluded pre-/pro-haptens



HTS-DCYA ASSAY

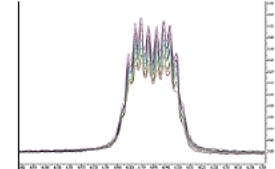
Cooper statistic analysis

	DCYA	Cys-DPRA*
True Positive	18	17
True Negative	9	8
False Positive	1	2
False Negative	5	0
Total articles	33	27

*Cys-DPRA results were taken from the literature

	DCYA	DPRA
Accuracy	81.8	92.6
Sensitivity	78.3	100.0
Specificity	90.0	80.0

- ✓ Based on the limited sample set, the HTS-DCYA results were comparable with the recently approved Cys-DPRA
- ✓ Less false positives due to limited auto-oxidization of the nucleophile



NMR-DCYA ASSAY

Spectroscopic method recently developed at UM based on nuclear magnetic resonance¹

Features

Ideal for the *direct quantification of reaction adducts* by NMR spectroscopy

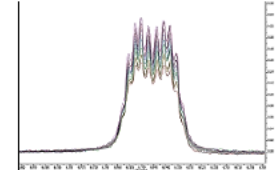
Advantages

- ✧ Multifaceted method allows monitoring of the depletion of test articles, nucleophile and/or formation of adducts
- ✧ Direct quantification of reactivity and can be applied to improve *in silico* predictions
- ✧ Ideal for *structural, molecular and mechanistic analysis*, to identify the reaction site in the presence of multiple mechanistic domains

Limitations

- One sample at a time, time-consuming and less sensitive than HTS-DCYA
- Pre- or pro- sensitizers may require chemical/metabolic activation before screening

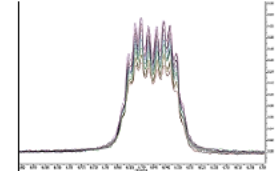
¹U.S. Provisional Application Serial No. 62/017,586; Manuscript under review, *Chem Res Toxicol*



NMR-DCYA ASSAY

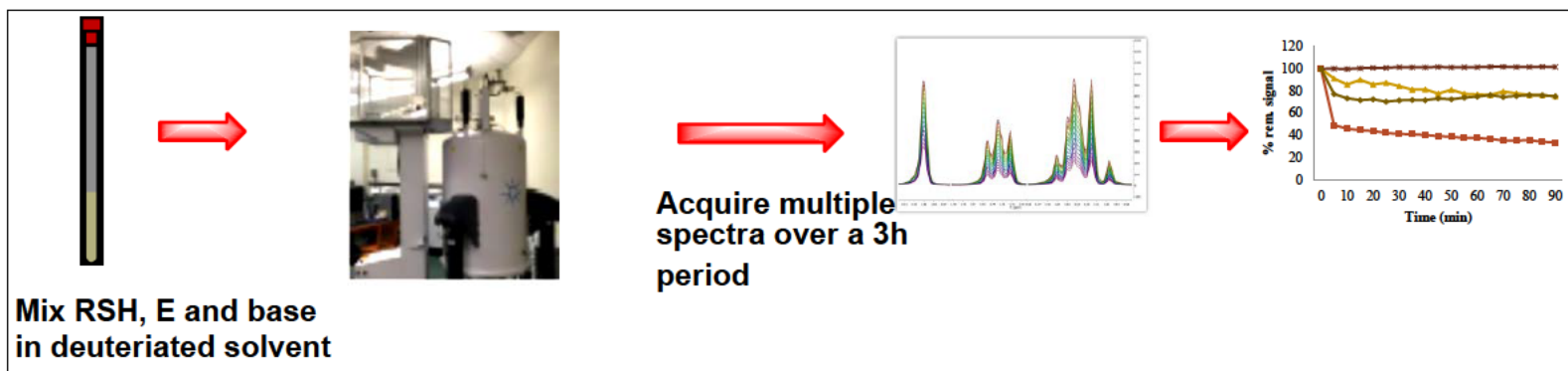
Description

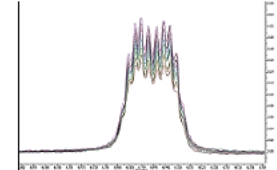
- *In chemico* spectroscopic method based on the quantification of test article/nucleophile depletion and/or formation of adduct (test article-DCYA) by nuclear magnetic resonance
- The candidate sensitizer (test article) is mixed with the model nucleophile (*e.g.* DCYA). The reaction is monitored by acquiring ^1H -NMR spectrum at regular intervals for 3 h
- The reaction is quantified by calculating the variation of distinctive resonance signal(s) of the sensitizer and/or nucleophile and/or by monitoring signals corresponding to the adduct
- The rate of depletion is directly proportional to potency of the given test article



NMR-DCYA ASSAY

Flow diagram of NMR method



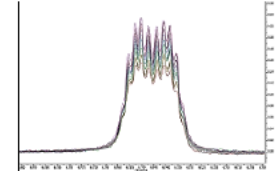


NMR-DCYA ASSAY

Method validation

- A total of 17 compounds* were chosen for the validation of method described in the previous slides
- Pre-/pro-haptens, non-sensitizers, weak, moderate and strong/extreme sensitizers have been included

*The list of compounds used for the method validation included *p*-Benzoquinone, Ethyl acrylate, *p*-Hydroquinone, 3-Hydroxytyrosol, Cinnamaldehyde, Safranal, Perillaldehyde, Citral, L-Carvone, Coumarin, Nootkatone, Curcumin, Massoia lactone, 2-pentenal, Parthenolide, Costunolide, Alantolactone



NMR-DCYA ASSAY

Method validation*

	LLNA/PATCH TEST	NMR-DCYA
Non-sensitizer	1	2
Weak	0	2
Moderate	5	2
Strong/extreme	5	5
Tot. positive	10/11	9/11
Tot. negative	1/11	2/11

*Pre-/pro-hapten were excluded from the statistical analysis

IN VITRO

hCLAT

Features

- *In vitro* method based on expression of CD86 and CD54
- THP-1 cell lines are used as a substitute for human dendritic cells (DC)

Advantages

- ✧ *In vitro* assay
- ✧ Recently validated by European Regulatory agencies

Limitations

- Costly
- Time-consuming
- Cell based assay, testing cytotoxic component(s) is problematic
- Low throughput (3 samples/assay)

IN VITRO

hCLAT

Description

- Dendritic cells (DC) activation is one of the major steps of the induction phase of skin sensitization
- During the process, DC change from antigen processing to antigen presenting cells and exhibit up regulation of expression CD86 and CD54
- The hCLAT method is based on the expression of CD86 and CD54 protein markers on the surface of the human monocytic leukemia cell line (THP-1), following exposure to test chemicals

IN VITRO

hCLAT

Experimental design



Pre-culture THP-1 cells for 48-72 hours

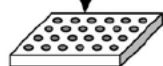
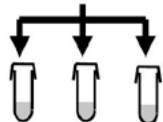


Plate (1×10^6 cells/well) in 24-well plate, treat with test chemical for 24 hours



Harvest cells, wash and block FcR (0.01% Globulins) for 15 min.



Divide cells into 3 aliquots, stain with FITC-conjugated monoclonal antibodies (isotype control, CD86, CD54) for 30 min.



Analyze by flow cytometry - mean fluorescence intensity of CD86 and CD54, cell viability by propidium iodide exclusion.

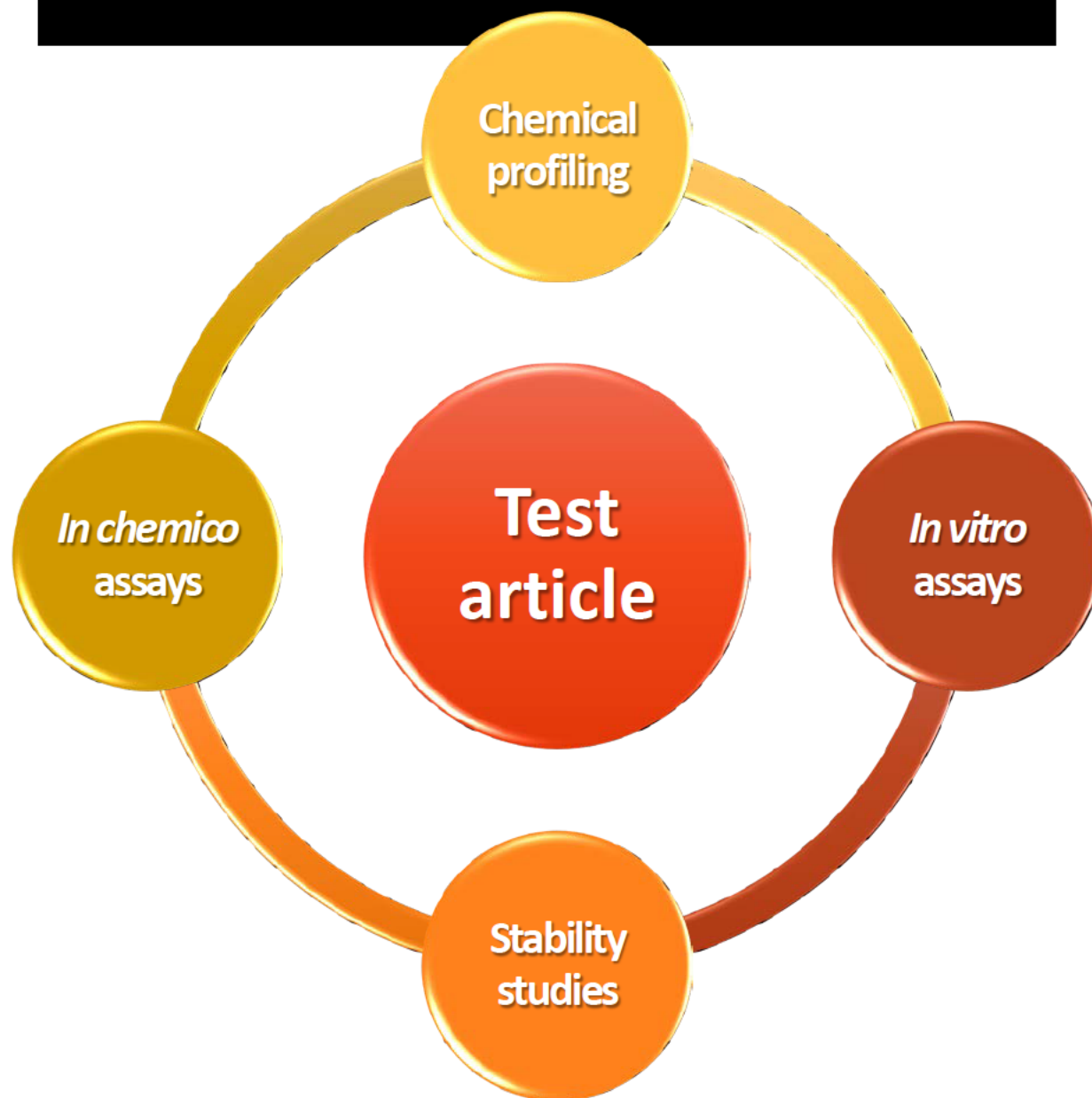
Two of three independent measurements at any dose should exceed the positive criteria (CD86 >150% or CD54 >200%) in order to be judged as positive.

Status

- The method has been optimized
- Validation with know sensitizers is in progress

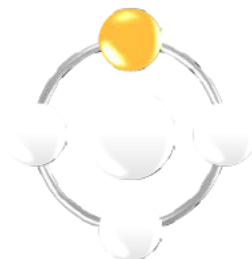
RISK ASSESSMENT OF SENSITIZERS IN SKIN CARE PRODUCTS

26 FRAGRANCE ALLERGENS



26 FRAGRANCE ALLERGENS

CHEMICAL PROFILING

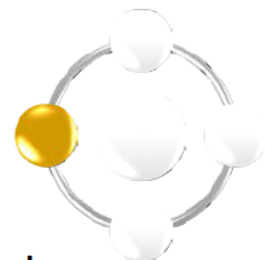


- Is there any known Substance of Concern (SoC)?
 - ✓ Analytical investigation for 26 fragrance allergens
 - ✓ Assessment of the presence of other suspects allergens (see extended list of 80 chemicals)
- Is there any potential *source* of SoC?
 - ✓ Presence of congeneric groups?
 - ✓ Chemically related compounds?
 - ✓ Stability issues?

26 FRAGRANCE ALLERGENS

IN CHEMICO ASSAYS

- Is there any known/unknown potential SoC?
 - ✓ Assessment of the sensitization potential using chemical reactivity based assays
- How many SoC are present?
 - ✓ Assessment of mixtures for the overall sensitization potential in relationship to the major components
 - ✓ Formulation effects?



26 FRAGRANCE ALLERGENS

STABILITY STUDIES

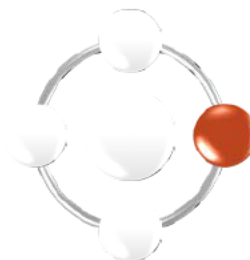
- Is there any chemical activation?
 - ✓ Air oxidization?
 - ✓ Light stability?
 - ✓ Reactive species formation?
 - ✓ pH stability?
- How many SoC are present?
 - ✓ Assessment of mixtures for the overall sensitization potential in relationship to the major components
 - ✓ Formulation effects?



26 FRAGRANCE ALLERGENS

IN VITRO STUDIES

- Identified selected articles and SoC can be further investigated using *in vitro* assays available at UM
 - ✓ hCLAT
- Integrated Strategy (both *in chemico* and *in vitro* data for same test article) for estimating the potency of potential allergens
 - ✓ Data integration and harmonization?
 - ✓ Better predictability and reliability?
- Inter-laboratory validation for both *in chemico* methods



BOTANICALS FOR SKIN CARE

Current status

- ✓ *In chemico* methods developed at UM are currently under optimization for the screening of mixtures, plant extracts and botanicals of cosmetic relevance and pre-haptens
- ✓ A list of fragrances and natural constituents currently under evaluation includes limonene, linalool, α -terpinene, γ -terpinene, terpinolene, *p*-cymene, ascaridole, jasmone, citral, coumarin, cinnamaldehyde
- ✓ A list of plant extracts currently under evaluation with UM_HTS method includes lavender, German and Roman chamomile, *Arnica montana*, *Taraxacum officinale*, *Cinnamomum* spp., *Citrus* spp., *Cymbopogon* spp., *Calendula officinalis*, *Tanacetum parthenium*, *Hamamelis virginiana*, *Pogostemon cablin*



Scientific Committee on Consumer Safety

SCCS

OPINION
on
Fragrance allergens in cosmetic products

The SCCS adopted this opinion at its 15th plenary meeting

of 26-27 June 2012

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

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Summary

Contact allergy to fragrance ingredients may develop following skin contact with a sufficient amount of these substances, often through the use of cosmetic products. Contact allergy is an altered specific reactivity in the immune system, which entails recognition of the fragrance allergen(s) in question by immune cells. Contact allergy, which *per se* is a latent condition, i.e. without visible signs or symptoms, persists lifelong. Upon each re-exposure to sufficient amounts of the allergen(s) eczema develops (allergic contact dermatitis), which typically will involve the face, the armpits and/or the hand(s). The disease can be severe and generalised, with a significant impairment of quality of life and potential consequences for fitness for work.

Around 16% of eczema patients in the European population are sensitised to fragrance ingredients. From studies performed on sectors of the population it can be estimated that the frequency of contact allergy to fragrance ingredients in the general population in Europe is 1-3%. The overall trend of fragrance allergy has been stable during the last 10 years, as some causes of fragrance allergy have decreased and others increased.

Most individuals with contact allergy to fragrance ingredients are aware that they cannot tolerate scented products on their skin and are often able to specifically name product categories that initiated their disease. In this context colognes, eau de toilette, deodorants and lotions are named significantly more often by fragrance allergic eczema patients than by patients without fragrance contact allergy.

Commercially available fragrances and other scented cosmetic products can provoke allergic contact dermatitis under patch test as well as simulated use conditions.

Appropriate diagnostic procedures and patient information are cornerstones in secondary prevention of contact allergy. The SCCNFP identified in 1999 a set of 26 fragrance allergens with a well-recognised potential to cause allergy, for which information should be provided to consumers about their presence in cosmetic products.

This listing has shown to be important in the clinical management of patients who are allergic to one or more of these 26 fragrance chemicals. Listing of the 26 fragrances has also been shown to be beneficial for patients with contact allergy to one or more of the fragrance chemicals, because these are identified on the ingredient listings of cosmetic products, and can thus be avoided.

The present opinion updates the SCCNFP opinion with a systematic and critical review of the scientific literature to identify fragrance allergens, including natural extracts, relevant to consumers. Clinical, epidemiological and experimental studies were evaluated, as well as modelling studies performed, to establish lists of (i) established fragrance allergens, (ii) likely fragrance allergens and (iii) possible fragrance allergens.

The studies since the SCCNFP Opinion on fragrance allergy in consumers confirm that the fragrance allergens identified by SCCNFP in 1999 are still relevant fragrance allergens for consumers from their exposure to cosmetic products. The review of the clinical and experimental data published since then shows that many more fragrance substances have been shown to be sensitisers in humans. Based on the clinical experience alone, 82 substances can be classified as established contact allergens in humans, 54 single chemicals and 28 natural extracts. Of these, 12 chemicals and 8 natural extracts were found to pose a high risk of sensitisation to the consumer, considering the high number of reported cases. In particular one ingredient stood out, hydroxyisohexyl 3-cyclohexene carboxaldehyde, having been the cause of more than 1500 reported cases since the 1999 opinion.

Moreover, animal experiments indicate that additional fragrance substances can be expected to be contact allergens in humans, although human evidence is currently lacking. Additionally, limited *in vivo* evidence together with Structure-Activity Relationship analysis suggests that other fragrance ingredients may also be a cause of concern with regard to their potential of causing contact allergy in humans.

The review also lists fragrance substances that can act as prehaptenes or prohaptens, forming new or more potent allergens by air oxidation and/or metabolic activation. Such

activation processes are of concern as they increase the risk of sensitisation and also the risk for cross reactivity between fragrance substances. In addition to known prehapten fragrance substances, the SCCS performed SAR analyses to identify fragrance substances with structural alerts that indicate that they are possible prehapten. While in the case of prohapten the possibility of becoming activated is inherent to the molecule and cannot be avoided, the activation of prehapten can be prevented by appropriate measures.

The SCCS examined available elicitation dose-response data to decide whether safe thresholds can be established for the fragrance allergens of concern, i.e. those found to pose a high risk of sensitisation to consumers. The SCCS considers that thresholds based on elicitation levels in sensitised individuals will be sufficiently low to protect both the majority of sensitised individuals as well as most of the non-sensitised consumers from developing contact allergy. As data from human dose elicitation experiments are very limited in several respects, no levels that could be considered safe for the majority of contact allergic consumers could be established for individual substances. The studies available, however, indicate that a general level of exposure of up to 0.8 µg/cm² (0.01% in cosmetic products) may be tolerated by most consumers, including those with contact allergy to fragrance allergens. The SCCS is of the opinion that this level of exposure (up to 0.01%) would suffice to prevent elicitation for the majority of allergic individuals, unless there is experimental or clinical substance-specific data allowing the derivation of individual thresholds.

It was not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist and the model providing the general threshold (0.01%) has been based on individual chemicals only. However the SCCS considers that the maximum use concentration applies to the identified chemicals both if added as chemicals or as an identified constituent of a natural ingredient. This will also reduce the risk of sensitisation and elicitation from natural extracts.

The suggested general threshold, although limiting the problem of fragrance allergy in the consumer significantly, would not preclude that the most sensitive segment of the population may react upon exposure to these levels and does not remove the necessity for providing information to the consumer concerning the presence of the listed fragrance substance in cosmetics.

In the case of hydroxyisohexyl 3-cyclohexene carboxaldehyde, the SCCP had recommended limiting the concentration in cosmetics to 200 ppm. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized.

The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in products for the consumer. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents. The SCCS is of the opinion that the presence of the two constituents, chloroatranol and atranol, in cosmetic products are not safe.

1. Background

As a result of the public consultation on perfumery materials, which ended on 27 January 2007, there were further requests and information on important and/or frequently used allergens other than those proposed for regulation, such as farnesol, citral, linalool and hydroxyisohexyl-3-cyclohexenecarboxaldehyde. These substances were not part of the consultation, but they all belong to the 26 fragrance substances which should be labelled when present in cosmetic products under certain conditions.

The 26 fragrance substances were introduced into annex III of the Cosmetics Directive by the 7th amendment (2003/15/EC) on the basis of the SCCNFP draft opinion (SCCNFP/0017/98) published on 30 September 1999 for public consultation and the final opinion adopted by the SCCNFP during the plenary session of 8 December 1999.

Thirteen of the allergenic fragrance substances listed in this opinion have been frequently reported as well-recognised contact allergens in consumers and are thus of most concern; 11 others are less well documented. See the lists below from the opinion.

List A: *Fragrance chemicals, which according to existing knowledge, are most frequently reported and well-recognised consumer allergens.*

Common name	CAS number
Amyl cinnamal	122-40-7
Amylcinnamyl alcohol	101-85-9
Benzyl alcohol	100-51-6
Benzyl salicylate	118-58-1
Cinnamyl alcohol	104-54-1
Cinnamal	104-55-2
Citral	5392-40-5
Coumarin	91-64-5
Eugenol	97-53-0
Geraniol	106-24-1
Hydroxycitronellal	107-75-5
Hydroxymethylpentyl-cyclohexenecarboxaldehyde	31906-04-4
Isoeugenol	97-54-1

List B: *Fragrance chemicals, which are less frequently reported and thus less documented as consumer allergens.*

Common name	CAS number
Anisyl alcohol	105-13-5
Benzyl benzoate	120-51-4
Benzyl cinnamate	103-41-3
Citronellol	106-22-9
Farnesol	4602-84-0
Hexyl cinnamaldehyde	101-86-0
Lilial	80-54-6
d-Limonene	5989-27-5
Linalool	78-70-6
Methyl heptine carbonate	111-12-6
3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	127-51-5

Furthermore, two fragrances (natural mixtures) were added

Common name	CAS number
Oak moss	90028-68-5
Tree moss	90028-67-4

At the time there were insufficient scientific data to allow for the determination of dose-response relationships and/or thresholds for these allergens. Nevertheless, in a pragmatic administrative decision the limits of 0.01 and 0.001% were set, for rinse-off and leave-on products respectively.

Scientific information of both a general and a specific nature has been submitted to DG ENTR in order to ask the SCCS for a revision of the 26 fragrances with respect to further restrictions and possible even delisting.

2. Terms of reference

- 1. Does the SCCS still consider that the fragrance allergens currently listed in Annex III, entries 67-92, for labelling purposes represent those fragrance ingredients that the consumer needs to be made aware of when present in cosmetic products?*
- 2. Can the SCCS establish any threshold for their safe use based on the available scientific data?*
- 3. Can the SCCS identify substances where processes (e.g. metabolism, oxidation and hydrolysis) may lead to cross-reactivity and new allergens which are relevant for the protection of the consumer?*

3. Introduction

Fragrance ingredients

Fragrance and flavour substances are organic compounds with characteristic, usually pleasant, odours. They are ubiquitously used in perfumes and other perfumed cosmetic products, but also in detergents, fabric softeners, and other household products where fragrance may be used to mask unpleasant odours from raw materials. Flavourings are used in foods, beverages, and dental products. Fragrance substances are also used in aromatherapy and may be present in herbal products, and used as topical medicaments for their antiseptic properties.

Contact allergy to fragrance ingredients occurs when an individual has been exposed, on the skin, to a sufficient degree of fragrance contact allergens. Contact allergy is a life-long, specifically altered reactivity in the immune system. This means that once contact allergy is developed, cells in the immune system will be present which can recognise and react towards the allergen. As a consequence, symptoms, i.e. allergic contact dermatitis, may occur upon re-exposure to the fragrance allergen(s) in question. Allergic contact dermatitis is an inflammatory skin disease characterised by erythema, swelling and vesicles in the acute phase. If exposure continues it may develop into a chronic condition with scaling and painful fissures of the skin. Allergic contact dermatitis to fragrance ingredients is most often caused by cosmetic products and usually involves the face and/or hands. It may affect fitness for work and the quality of life of the individual.

Fragrance contact allergy has long been recognised as a frequent and potentially disabling problem. Prevention is possible as it is an environmental disease and if the environment is modified (e.g. by reduced use concentrations of allergens), the disease frequency and severity will decrease. Ingredient information is a cornerstone in the prevention of allergic contact dermatitis, as knowledge about the allergens which a patient has been exposed to is crucial for including the right substances in the allergy test, and for subsequent information on avoidance of re-exposure. However, the labelling rules in the Cosmetics Directive 76/768/EEC stipulated that perfume and aromatic compositions and their raw materials shall be referred to by the word "perfume" or "aroma", rather than being labelled individually. This is the reason why the SCCNFP in their opinion SCCNFP/0017/98 (1) identified 26 fragrance allergens for which information should be provided to consumers concerning their presence in cosmetic products. This was implemented in the Cosmetics Directive as individual ingredient labelling of the 26 fragrance allergens (Annex III, entries 67-92). However, safe use concentrations of these fragrances in cosmetic products had not yet been determined and much new evidence concerning fragrance allergy has been published since the 1999 opinion. The present request to review the list of recognised fragrance allergens which the consumer needs to be made aware of, to indicate thresholds for their safe use and to consider possible modification of allergens by metabolism and autoxidation, required a thorough review of all relevant scientific data. This includes both published scientific literature as well as unpublished scientific information on fragrances from the industry. The International Fragrance Association (IFRA), as representative of the fragrance industry, was contacted to provide relevant unpublished scientific data on fragrance ingredients. This information, together with the up-to-date published scientific literature, has been critically reviewed for the present SCCS opinion. The relevant data gaps are identified and recommendations for research addressing these gaps are made.

4. Clinical aspects of contact allergy to fragrance ingredients

4.1. Spectrum of reactions

Adverse reactions to fragrances in perfumes and in fragranced cosmetic products include allergic contact dermatitis, irritant contact dermatitis, photosensitivity, immediate contact reactions (contact urticaria), and pigmented contact dermatitis. Airborne and connubial contact dermatitis occur.

4.1.1. Allergic contact dermatitis

Mechanism

Allergic contact dermatitis (ACD) depends primarily on the activation of allergen-specific T-cells. In allergic contact dermatitis, a distinction is made between induction (sensitisation) and elicitation phases. A useful review is available (2).

The induction phase includes the events following initial contact with the allergen and is complete when the individual is sensitised and capable of giving a positive allergic contact dermatitis reaction.

The elicitation phase begins upon re-exposure to the allergen (challenge) and results in clinical manifestation of allergic contact dermatitis.

The entire process of the induction phase requires ca. 10 days to several weeks, whereas an elicitation phase reaction develops within 1–2 days.

Most contact allergens are small, chemically reactive compounds. As these compounds are too small to be directly immunogenic, they act as haptens; i.e. they react with higher molecular weight epidermal and/or dermal biomolecules to form immunogenic adducts. It is usually considered that the biomolecules involved are free or membrane bound proteins, which react via nucleophilic thiol, amino, and hydroxyl groups.

Dendritic cells (DCs) and the local tissue microenvironment are crucial factors in the development of ACD. Langerhans cells (LCs), as epidermal DCs, and dermal DCs are pivotal for the sensitisation and the elicitation phases of ACD. During sensitisation, DCs react with the immunogenic complexes by interaction with neighbouring keratinocytes, migration to the local draining lymph nodes and the priming of naïve T-cells. These reactions are mediated by inflammatory cytokines, chemokines and adhesion molecules. Antigen specific effector T-cells are then recruited into the skin upon contact with the same hapten (elicitation). Following their recruitment these T-cells are activated by antigen-presenting skin cells, including LCs, dermal DCs and keratinocytes, and macrophages.

Although most allergens can form hapten–carrier complexes directly, some need activation, e.g. by enzyme-induced metabolic conversion or abiotic oxidation. Such compounds are termed prohaptens and prehaptens, respectively, and are discussed in more detail in chapter 5. Well known examples of prehaptens and prohaptens are limonene and eugenol. Reduced enzyme activity in certain individuals, related to genetic enzyme polymorphisms, may give an increased or reduced risk of sensitisation to prohaptens (that need enzymatic activation) in certain individuals or populations.

Once sensitised, individuals can develop allergic contact dermatitis upon re-exposure to the contact allergen. Positive patch test reactions mimic this process of allergen-specific skin hyper-sensitivity. Skin contact induces an inflammatory reaction that is maximal within 2–3 days and, without further allergen supply, then declines.

Overview of clinical features

Perfumes and deodorants are the most frequent sources of sensitisation to fragrance ingredients in women, while aftershave products and deodorants are most often responsible in men (3). Thereafter, eczema may appear or be worsened by contact with other

fragranced products such as cosmetics, toiletries, household products, industrial contacts and flavourings.

Contact allergy to a particular product or chemical is established by means of diagnostic patch testing. When patients with suspected allergic cosmetic dermatitis are investigated, fragrances are identified as the most frequent allergens, not only in perfumes, after-shaves and deodorants, but also in other cosmetic products. Evaluation of perfume allergy may be difficult; a perfume compound may consist of ten to > 300 basic components selected from about 2500 materials.

Between 6 and 14% of patients routinely tested for suspected allergic contact dermatitis react to a standard indicator of fragrance allergy, the Fragrance Mix I (4), see also chapter 4.3.2. When tested with ten popular perfumes, 6.9% of female eczema patients proved to be allergic to them (5) and 3.2–4.2% were allergic to fragrances from perfumes present in various cosmetic products (6). The finding of a positive reaction to the Fragrance Mix I should be followed by a search for its relevance, i.e. is fragrance allergy the cause of the patient's current or previous complaints, or does it at least contribute to it? Between 50 and 65% of all positive patch test reactions to the mix are relevant. Sometimes, correlation with the clinical picture is lacking and many patients appear to tolerate perfumes and fragranced products without problems (7). This may be explained by: a) irritant (false-positive) patch test reactions to the mix; b) the absence of relevant allergens in those products; and c) the concentration being too low to elicit clinically visible allergic contact reactions. Contact allergy to fragrances often causes dermatitis of the hands (and aggravation of), face and neck, axillae and patches in areas where perfumes are dabbed on such as behind the ears, upper chest, elbow flexures and wrists. Depending on the degree of sensitivity and exposure, the severity of dermatitis may range from mild to severe with dissemination (8) [pp 158–170].

Clinical studies have shown a highly significant association between reporting a history of visible skin symptoms from using scented products and a positive patch test to the Fragrance Mix I (9). Provocation studies with perfumes and deodorants have also shown that fragrance-mix-positive eczema patients often react to use-tests with the products. Subsequent chemical analysis of such products has detected significant amounts of one or more Fragrance Mix I ingredients, confirming the relevance of positive patch tests to the Fragrance Mix I in these patients (5, 10).

Hands

Contact sensitisation may be the primary cause of hand eczema, or may be a complication of irritant or atopic hand eczema. The number of positive patch tests has been reported to correlate with the duration of hand eczema, indicating that long-standing hand eczema may often be complicated by sensitisation (11). The most common contact allergies in patients with hand eczema are metals, the Fragrance Mix, *Myroxylon pereirae*, and colophonium (12).

Fragrance allergy may be a relevant problem in patients with hand eczema; perfumes are present in consumer products to which their hands are exposed (13). A significant relationship between hand eczema and fragrance contact allergy has been found in some studies based on patients investigated for contact allergy (14). However, hand eczema is a multi-factorial disease and the clinical significance of fragrance contact allergy in (severe) chronic hand eczema may not be clear. A review on the subject has been published (15).

Axillae

Bilateral axillary dermatitis may be caused by perfume in deodorants and, if the reaction is severe, it may spread down the arms and to other areas of the body (8) [pp 158–170]. In individuals who consulted a dermatologist, a history of such first-time symptoms was significantly related to the later diagnosis of perfume allergy (9).

Face

Facial eczema is an important manifestation of fragrance allergy from the use of cosmetic products (16). In men, aftershave products can cause an eczematous eruption of the beard area and the adjacent part of the neck (8) [pp 158–170], and men using wet shaving as opposed to dry have been shown to have an increased risk of 2.9 of being fragrance allergic (17).

4.1.2. Irritant reactions (including contact urticaria)

Irritant effects of some individual fragrance ingredients, e.g. citral (18, 19), are known. Irritant contact dermatitis from perfumes is believed to be common, but there are no existing investigations to substantiate this (7). Many more people complain about intolerance or rashes to perfumes/perfumed products than are shown to be allergic by testing (9). This may be due to irritant effects or inadequate diagnostic procedures.

Fragrances may cause a dose-related contact urticaria of the non-immunological type (irritant contact urticaria). Cinnamal, cinnamic alcohol, and *Myroxylon pereirae* are well recognised causes of contact urticaria, but others, including menthol, vanillin and benzaldehyde have also been reported (20). The reactions to *Myroxylon pereirae* may be due to cinnamates (21).

A relationship to delayed contact hypersensitivity was suggested (22), but no significant difference was found between a fragrance-allergic group and a control group in the frequency of immediate reactions to fragrance ingredients (20), in keeping with a non-immunological basis for the reactions seen.

4.1.3. Pigmentary anomalies

The term “pigmented cosmetic dermatitis” was introduced in 1973 for what had previously been known as melanosis faciei feminae when the mechanism (type IV allergy) and causative allergens were clarified (23). It refers to increased pigmentation, usually on the face/neck, often following sub-clinical contact dermatitis. Many cosmetic ingredients were patch tested at non-irritant concentrations and statistical evaluation showed that a number of fragrance ingredients were associated: jasmine absolute, ylang-ylang oil, cananga oil, benzyl salicylate, hydroxycitronellal, sandalwood oil, geraniol, geranium oil (24).

4.1.4. Photo-reactions

Musk ambrette produced a considerable number of allergic photocontact reactions (in which UV-light is required) in the 1970s (25) and was later banned from use in the EU. Nowadays, photoallergic contact dermatitis is uncommon (26). Furocoumarins (psoralens) in some plant-derived fragrance ingredients caused phototoxic reactions with erythema followed by hyperpigmentation resulting in Berloque dermatitis (8) [pp 417–432]. There are now limits for the amount of furocoumarins in fragrance products. Phototoxic reactions still occur but are rare (27).

4.1.5. General/respiratory

Fragrances are volatile and therefore, in addition to skin exposure, a perfume also exposes the eyes and naso-respiratory tract. It is estimated that 2–4% of the adult population is affected by respiratory or eye symptoms by such an exposure (28). It is known that exposure to fragrances may exacerbate pre-existing asthma (29). Asthma-like symptoms can be provoked by sensory mechanisms (30). In an epidemiological investigation, a significant association was found between respiratory complaints related to fragrances and contact allergy to fragrance ingredients, in addition to hand eczema, which were independent risk factors in a multivariate analysis (31).

4.2. Patch testing

The diagnosis of contact sensitisation (or contact allergy – regarded here as synonymous) as the immunological alteration underlying allergic contact dermatitis is made by patch testing. This diagnostic tool involves the standardised application of small doses of a set of potential or individually suspected allergens for a period of 1 day or, mostly, 2 days. In the following days, exposed skin sites are checked for the occurrence of allergic reactions, which morphologically mimic allergic contact dermatitis occurring elsewhere, after exposure to culprit products. International guidelines for the application, reading and interpretation of the patch test exist (32). The present brief section does not intend to reiterate all technical and scientific aspects, but to outline some aspects of diagnostic patch testing which are often misunderstood (for a recent comment see also (33)).

- The patch test identifies whether the patient has contact allergy to a substance, but cannot contribute information on the clinical relevance of that contact allergy for the eczema that led to consultation and to patch testing (see 4.4.1).
- Exposure conditions of the patch test (one-time, prolonged occlusive application, usually in petrolatum or water, of a single substance) have been optimised to achieve above diagnostic aim, and thereby have nothing in common with exposures which lead to sensitisation and elicitation of allergic contact dermatitis. These are normally repetitive, often over weeks, months or years, non-occlusive, and to much lower concentrations and doses/area, respectively, but possibly on damaged or inflamed skin. In fact, the repeated open application test (ROAT), which is sometimes used after a positive patch test of uncertain validity to verify that contact allergy indeed exists mimicks these day-to-day exposure conditions, and typically involves single dosings which are a small fraction of the one-time patch test dose (see 11).
- It is self-evident that such (repeated, low-level) exposures must have occurred and have culminated in an adaptive immune response – therefore it is axiomatic that the substance involved is a skin sensitizer in humans (33).
- Repeated patch testing, which is a relatively rare event, does not contribute significantly to contact allergy (to fragrance allergens).
- Most allergen test preparations, and certainly those that are included in international baseline series, have evolved from studies critically (re-) appraising their diagnostic validity, i.e., sensitivity and specificity. Notwithstanding this, false-positive and false-negative reactions do occur (as with any diagnostic tool). While in the individual case such diagnostic misclassification may have unfortunate consequences, it will hardly impair epidemiological estimates of contact allergy frequency – at least as long as a reasonable balance between false-positive and false-negative reactions is achieved.

4.3. Epidemiology of fragrance allergy

4.3.1. Substances used for screening of contact allergy to fragrance ingredients

A fragrance formula may consist of ten to 300 or more different ingredients. The CosIng database lists 2587 ingredients used for perfuming¹, as well as several other materials classified as odour “masking” agents, which is equivalent with regard to allergy. A mixture of seven fragrance chemicals and one natural extract, which have been identified as major fragrance allergens in the past (34), are used for diagnosing contact allergy to fragrance

¹ <http://ec.europa.eu/enterprise/cosmetics/cosing/index.cfm?fuseaction=search.results&function=66&search>, last accessed 2009-10-14.

ingredients (Table 4-1). This mixture is called the Fragrance Mix (FM I) and is included in the standard patch test tray containing the most common allergens in Europe.

Table 4-1: Ingredients of Fragrance Mix I (FM I; 8% allergens in petrolatum).

Single constituent: INCI name (common name)	Conc. (%)
Amyl cinnamal (alpha-amyl cinnamal)	1
Cinnamyl alcohol (cinnamic alcohol)	1
Cinnamal (cinnamic aldehyde)	1
Eugenol	1
Geraniol	1
Hydroxycitronellal	1
Isoeugenol	1
Oak moss absolute (a natural extract; INCI: <i>Evernia prunastri</i>)	1
Sorbitan sesquioleate (added as an emulsifier)	5

Note: All single allergens of the above, when used for breakdown testing, are also in petrolatum.

However, due to the introduction of new fragrance ingredients (with allergenic potential), the above Fragrance Mix I was deemed not to be sufficient for the diagnosis of fragrance allergy. Thus, Fragrance Mix II was devised to supplement Fragrance Mix I in a European multicentre study (35, 36). Since then, FM II has been included in the European baseline series. Table 4-2 lists the ingredients of FM II. In addition to being tested in FM II, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) is also tested separately at 5% test concentration in the baseline series (37).

Table 4-2: Ingredients of Fragrance Mix II (FM II; 14% allergens in petrolatum).

Single constituent: INCI name (common name)	Conc. (%)
Citronellol	0.5
Citral	1
Coumarin	2.5
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)	2.5
Farnesol	2.5
Alpha-hexyl-cinnamal	5

Note: All single allergens of the above, when used for breakdown testing, are also in petrolatum.

Patch test results in patients and in population samples with these two screening mixes, and single allergens, will be presented and discussed in the following two sections.

4.3.2. Clinical epidemiology

For a number of reasons the bulk of the evidence regarding the frequency of contact allergy to fragrance ingredients relies on clinical data, i.e. the history, clinical presentation and test results of patients patch tested for suspected allergic contact dermatitis – in general, and not specifically due to fragrance ingredients. The frequency of contact allergy to fragrance ingredients (or other contact allergies, for that matter) cannot be related to the population

directly, as it is derived from a subgroup (of patients) selected for specific morbidity. Nevertheless, these data can be examined epidemiologically assuming a largely similar selection process: (i) across time in a given department; and (ii) between departments at any point of time. If the notion of similarity, and thus direct comparability, does not appear valid, adjustment or standardisation techniques can be employed to account for differences, e.g. the average age of patients in a time series on a (fragrance) allergen with age-associated risk of sensitisation. In this situation, changes in the age composition of the patients tested may confound a time trend. A distinction must be made between patch testing “consecutive” patients, i.e. all patients who are patch tested for suspected contact sensitisation, and “aimed” patch testing, i.e. application of allergens only in the subset of patients in whom exposure to the particular allergens of the applied “special series” is suspected. For any given allergen, the latter “aimed” approach will usually yield higher sensitisation prevalences than the testing of not-further-selected “consecutive” patients. Thus, information on the inclusion of an allergen either in a baseline series (tested in virtually all patients) or in a special series (applied in an aimed fashion) must be considered and is given in the following tables, where available in the cited references.

Notwithstanding the potential pitfalls of clinical data, they have proven useful in identifying emerging trends or persisting problems, and also in evaluating the effect of preventive action – either regarding the entire population, or subgroups thereof, such as certain occupations. Regarding the fragrance mixes (FM I and FM II) mentioned above, evidence regarding sensitisation frequencies published since 1999 will be outlined below, thus supplementing the data presented in the SCCNFP opinion on Fragrance Allergy in 1999 (1).

Fragrance Mix I ("Larsen Mix")**Table 4-3:** Results with screening agents for contact allergy to fragrance ingredients reported since 1999 in patients patch tested for suspected allergic contact dermatitis in Europe: Fragrance Mix "I" (see Table 4-1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS (§).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
Sweden (38)	Consecutive patients	2000	3790	6.9
Hungary (39)		1998-1999	3604	8.2 (7.3–9.1) [§]
Czech Republic (40)		1997-2001	12058	5.8 (5.4–6.2) [§]
Ljubljana, Slovenia (41)	Consecutive patients	1989-1998	6129	5.9 (5.3–6.5) [§]
Germany (42)	Consecutive IVDK patients	1996-2002	59298	11.3 (11.0–11.5) [§]
Germany (43)	Consecutive IVDK patients	2005-2008	36961	7.3 (7.0–7.6) [§]
Vienna, Austria (16)	Consecutive patients of one clinic	1997-2000	2660	9.1 (8.1–10.3) [§]
Groningen, Netherlands (44)	Patients (fragrance allergy suspected)	04/2005-06/2007	295	5.8 (3.4–9.1) [§]
The Netherlands (45)	Consecutive patients	09/1998-04/1999	1825	10.6 (9.2–12.1)
The Netherlands (46)	Patients (cosmetic allergy suspected)	1994-1998	757	14.8 (12.3–17.5) [§]
Leuven, Belgium (47)	Consecutive patients	1990-2005	10128	9.1 (8.6–9.7) [§]
Coimbra, Portugal (48)	Consecutive patients	07/1989-06/1999	2600	10.9 (9.7–12.2) [§]
Spain (49)	Consecutive patients	10/2005-06/2008	1253	4.5 (3.4–5.8) [§]
Sheffield, UK (50)	Consecutive patients	1994-1995	744	11.4 (9.2–13.9) [§]
St. John's, London, UK (51)	Consecutive patients	1980-2004	34072	7.7 (7.4–8.0) [§]
Copenhagen, Denmark (52)	Consecutive patients	1985-2007	16173	7.2 (6.8–7.6) [§]
ESSCA (53)	Consecutive patients	2002-2003	9663	7.1 (6.6–7.6) [§]
ESSCA (54)	Consecutive patients	2004	9941	7.6 (7.1–8.2) [§]
ESSCA (55)	Consecutive patients	2005-2006	18542	7.0 (6.6–7.4) [§]

Table 4-4: Results with screening agents for contact allergy to fragrance ingredients reported since 1999 in patients patch tested for suspected allergic contact dermatitis in non-European countries: Fragrance Mix "I" (see Table 4-1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS (§).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
South Korea (56)	Consecutive patients	04/2002–06/2003	422	9.7 (7.1–13.0) [§]
Lahore, Pakistan (57)	Dermatitis patients	2 years prior to 2002	350	7.7 (5.2–11.0) [§]
Manipal, India (58)	Dermatitis patients	1989-1998	1780	3.1 (2.3–4.0) [§]
Tel Aviv, Israel [§] (59)	Consecutive patients	1999-2000	943	8.5 (6.8–10.5) [§]
Tel Aviv, Israel (60)	Consecutive patients	1998-2004	2156	7.1 (6.1–8.3) [§]
Tehran, Iran (61)	Consecutive patients	2002-2004	250	4.0 (1.9–7.2) [§]
Ankara, Turkey (62)	Consecutive patients	1992-2004	1038	2.1 (1.3–3.2) [§]
Beijing, China (63)	Consecutive patients	2000-2003	378	15.9 (12.3–20.0) [§]
USA (Canada) (64)	Probably consecutive patients	2003	1603	5.9
NACDG 2009 (US and Canada) (65)	Consecutive patients	2005-2006	4439	11.5

Note: § Possibly included in (60).

Beyond the studies discussed above, regarding a time trend of sensitisation to FM I, a significant increase of positive results to FM I until 1998, and a significant drop thereafter has been noted in the IVDK study covering 1996 to 2002 (42). A similar drop from 1999 to 2007 has been observed in female, but not male patients from Copenhagen (52). In accordance with these findings, the prevalence of positive reactions to FM I doubled, or thereabouts, from 1989-1993 to 1994-1998 in Ljubljana, Slovenia (41).

Within Europe, a comparison between different countries and clinical departments is possible. An EECDRG study covering 1996-2000 found 9.7% positives to FM I (range: 5.0–12.6% in ten departments from seven European countries (66). A different European study, covering 10/1997-10/1998, found 11.3% (95% CI: 9.9–12.9%) positive reactions to FM 1 in 1,855 patients; the variation between centres was marked: Gentofte 8.2% vs. Leuven 23.0% as extremes (67). In the first study of the European Surveillance System on Contact Allergies (ESSCA), covering 2002 and 2003, 9663 patients were patch tested with FM I, overall yielding 7.1% positive reactions with marked variation between participating departments. In Dortmund, Germany, the minimum frequency of 3.7% was noted, while in Lahti, Finland, the highest prevalence, namely 10.4%, was found (53). Subsequently, in the year 2004, the overall prevalence was 7.6%, i.e. largely unchanged (54). In the most recent study by ESSCA, based on 2005/2006 PT data across Europe, significant differences were again noted, this time on the aggregated level of European regions, with FM I sensitisation being the least frequent in the Southern countries (4.8% [95% CI: 3.9–5.5%] age- and sex-standardised prevalence) vs. 7.7% (95% CI: 7.0–8.4%) in the central European departments, with the Finnish, Polish and Lithuanian departments (5.7% [95%

CI: 4.6 – 6.8%]) and the UK network (6.8% [95% CI: 6.3 – 7.3%]) in an intermediate position (55).

Fragrance Mix II

Table 4-5: Results with screening agents for contact allergy to fragrance ingredients reported since 1999 in patients patch tested for suspected allergic contact dermatitis: Fragrance Mix "II" (see Table 4-2). The FM II was only conceived in 2005, so results are still sparse). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ([§]).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
EU (35)	Six clinical depts.	10/2002-06/2003	1701	2.9 (2.2–3.9) [§]
IVDK, Germany (68)	Consecutive patients	01/2005-12/2008	35633	4.9 (4.7–5.1) [§]
Groningen, Netherlands (44)	Patients (fragrance allergy suspected)	04/2005-06/2007	227	9.3 (5.8–13.8) [§]
Leuven, Belgium (47)	Consecutive patients	2005 only	335	2.1 (0.8–4.3) [§]
Spain (49)	Consecutive patients	10/2005-06/2008	1253	0.6 (0.2–1.1) [§]
Denmark (69) on behalf of the DCDG, 2010	Consecutive patients	2005-2008	12302	4.5 (4.1–4.9) [§]

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported chemical causing fragrance allergy since the 1999 opinion on fragrance allergy. In total, reports of about 1500 cases have been published in the scientific literature (see section 7.1).

HICC was recognised as an allergen in 1995 (70) and later included in the new perfume mixture, Fragrance Mix II (71), which is routinely used for the diagnosis of perfume allergy, see above. Furthermore, it is recommended to test separately with HICC, because it is a very frequent allergen (37) and detects relevant fragrance sensitisation which would otherwise have been missed (49). In the studies performed in European dermatology clinics, 0.5-2.7% of eczema patients have been found to be allergic to HICC with the highest frequency in central Europe (55). For further details see Table 4-6.

Table 4-6: Results with fragrance contact allergy screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **HICC** (5% pet. if not stated otherwise). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ([§]).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
Lithuania (72)	Consecutive patients	04/2006-10/2008	816	0.9 (0.3–1.8) [§]
Spain (49)	Consecutive patients	10/2005-06/2008	852	0.8 (0.3–1.7) [§]
Germany (CH, AT) (73)	Consecutive patients	03/2000-02/2001	3245	1.9 (1.5–2.4) [§]

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI)
Germany (CH, AT) (74)	Consecutive patients	01/2003-12/2004	21325	2.4 (2.2–2.6) [§]
Germany (CH, AT) (68)	Consecutive patients	01/2005-12/2008	35582	2.3 (2.2–2.5) [§]
Belgium (47)	Consecutive patients	2002-2005	2901	2.1 (1.6–2.7) [§]
Denmark (69)	Consecutive patients	2005-2008	12302	2.4 (2.1–2.7) [§]
South Korea (56)	Consecutive patients	04/2002–06/2003	422	1.7 (0.6–3.4) [§]
USA, Canada (64)	Probably consecutive patients	2003	1603	0.4 (0.2–0.9) [§]

***Myroxylon pereirae* (Balsam of Peru)**

Myroxylon pereirae is a balm obtained from a Central American tree. It is used as a screening substance for fragrance allergy in Europe and other geographical areas. Although the crude balm is not used in Europe in cosmetics, extracts and distillates are used (75). This natural mixture has been employed as screening agent in the baseline series for many decades. Hence, a wealth of data is available; Table 4-7 summarises results of the past 10 years.

Table 4-7: Results with fragrance contact allergy screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: ***Myroxylon pereirae* resin** (Balsam of Peru) (25% pet.). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ([§]).

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI) [§]
Tel Aviv, Israel (59) #	Consecutive patients	1999-2000	943	6.6 (5.1–8.4) [§]
South Korea (56)	Consecutive patients	04/2002 – 06/2003	422	7.3 (5.1–10.3) [§]
Tel Aviv, Israel (60)	Consecutive patients	1998-2004	2156	3.6 (2.9–4.5) [§]
Manipal, India (58)	Dermatitis patients	1989-1998	1780	1.0 (0.5 – 1.5) [§]
Tehran, Iran (61)	Consecutive patients	2002-2004	250	2.4 (0.9–5.2) [§]
Sevilla, Spain (76)	Consecutive patients	2002-2004	863	5.8 (4.3–7.6) [§]
Ankara, Turkey (62)	Consecutive patients	1992-2004	1038	2.1 (1.3–3.2) [§]
Vienna, Austria (16)	Consecutive patients of one clinic	1997-2000	2660	5.4 (4.6–6.3) [§]
Czech Republic (40)	Consecutive patients	1997-2001	12058	7.3 (6.8–7.8) [§]
Spain (49)	Consecutive patients	10/2005-06/2008	1253	6.4 (5.1–7.9) [§]

Country (Ref.)	Population	Year(s)	No. tested	Crude % positive (95% CI) [§]
Copenhagen, Denmark (52)	Consecutive patients	1985-2007	16173	3.9 (3.6–4.2) [§]
Sweden (38)	Consecutive patients	2000	3790	6.5
Nine European countries (53)	Consecutive patients	2002-2003	9672	6.1
Germany, three Swiss and one Austrian Dept. (43)	Consecutive patients	2005-2008	36919	8.0 (7.7–8.3)
Ten depts. From seven EU countries (66)	Consecutive patients	1996-2000	26210	6.0
USA (Canada) (64)	Probably consecutive patients	2003	1603	6.6
NACDG 2009 (65)	Consecutive patients	2005-2006	4449	11.9

Oil of turpentine

This natural extract is not tested in all baseline series. It is considered as a minor screening allergen for fragrance contact allergy. Moreover, oil of turpentine is used as a raw material in perfumery (see Annex I). Table 4-8 summarises results of the past 10 years with patch testing of consecutive patients.

Table 4-8: Results with fragrance contact allergy screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **Oil of turpentine** (10% pet.) patients patch tested for suspected allergic contact dermatitis. If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ([§]).

Country	Population	Year(s)	No. tested	Crude % positive (95% CI) [§]
Lisbon, Portugal (77); virtually no .delta.-3-carene	Consecutive patients	1979-1983	4316	2.3 (1.9–2.8) [§]
Birmingham, UK (78)	Potters with occup. hand dermatitis	6 months; prior to 1996	24	14/4 pos. to "Indonesian turpentine"
Austria/Germany (IVDK) (79)	Consecutive patients	1992-1995	27658	0.47 (0.39–0.55) [§]
Austria/Germany (IVDK) (42)	Consecutive patients	1996-2002	59478	Annual prevalence 1.6 to 4.4%
Augsburg, Germany (80)	Population sample	1998	1141	1.2% (on population level!)
Europe (ESSCA) (53)	Consecutive patients	2002/03	3767	1.6%
Austria/Germany/Switzerland (IVDK) (43)	Consecutive patients	2005-2008	37163	1.8%

An “overall burden” of fragrance contact allergy, in terms of the prevalence of contact allergy to at least one of the up-to-five screening allergens present in the baseline series (FM I, FM II, HICC, *Myroxylon pereirae*, oil of turpentine) has not been given in the published studies. A re-analysis of data from the two published studies of the IVDK (43, 68), covering central Europe from 2005 to 2008 (Germany, Austria and Switzerland), yielded an estimate of such overall prevalence of 16.2% (95% CI: 15.8-16.6%) (IVDK technical report, 2011-11-18).

4.3.3. Population-based epidemiology

In principle, the examination of a representative sample of the population is the most valid approach for estimating disease frequency, as there is no systematic selection process. However, in practice, participation of much less than 70% of those approached introduces the possibility of self-selection and thus of biased morbidity (or risk) estimates. Moreover, the resources needed prohibit regular, e.g. yearly, patch test studies in a sample of several thousand persons. For these reasons few studies exist (see Table 4-9).

A Swedish study of hand eczema in an industrial city showed that among 1,087 individuals recruited from the general population with symptoms of present or previous hand eczema, 5.8% were positive to the Fragrance Mix (81). In Denmark, Fragrance Mix sensitivity was found in 1.1% (0.3-2.1%) of 567 persons drawn as a sample from the general Danish population; only nickel sensitivity was more prevalent (82). In Italy, female patients with hand eczema caused by contact with detergents were patch tested. Of 1100 women, 3.1% reacted to Fragrance Mix I (83). A control group of 619 female patients with no eczema disease were also patch tested; 1.3% were positive to the Fragrance Mix (83). On the other hand, in a sample of 593 healthy Italian recruits, only three positive reactions (0.50%) to FM I were observed (84). Among Danish school children, 14-15 years of age, fragrance contact allergy was detected in 1.8% by patch testing with Fragrance Mix I (85). A study of 85 American student nurses showed that 15 (17.6%) had a positive reaction to Fragrance Mix I; 12 of the individuals also had a positive history of contact dermatitis (86). In this study the concentration of Fragrance Mix I was 16% as opposed to the currently recommended concentration of 8% and the study included only young females. Both of these factors may have contributed to the high prevalence of fragrance sensitivity found.

In 1990, 1998 and 2006, samples of the Danish adult population living in the Copenhagen area were patch tested with the European baseline series. In total 4299 individuals aged 18-69 years (18-41 years only in 1998) completed a pre-mailed questionnaire and were patch tested with FM I and *Myroxylon pereirae* (82, 87, 88). In 1990, 1.1% were found positive to FM I and in 2006, 1.6% were positive, which means no general change. However, when the age group of 18-41 years was analysed, the prevalence of FM I sensitisation followed an inverted V-pattern among women, i.e. an increase from 0.7% in 1990 to 3.9% in 1998, followed by a decrease to 2.3% in 2006. The participation rate varied in the three samples from 71.5% in 1990 to 52.4% in 1998, and to 43.7% in 2006 (82, 87, 88).

Contact sensitisation to FM I is strongly age related, with the relative risk more than doubling in the older age groups, compared to younger PT patients. This has been found in both bivariate (89) and adjusted multifactorial analyses (90). Hence, in older samples of the population, the prevalence of contact allergy to fragrance ingredients in general, and to FM I in particular, can be expected to be higher than in younger samples. From this background, the strikingly high prevalence observed in the MONICA/KORA allergy study in Augsburg, Germany (see Table 4-9) (80), may be explained, together with some residual confounding from the rather complex sampling process.

Table 4-9: Results from patch testing with Fragrance Mix I in different population based groups.

Country (Ref.)	Population	Year(s)	No. tested	% positive (95% CI)
Italy (83)	Females without eczema	Not given	619	1.3
Italy (84)	Male recruits	Not given	593	0.50
Denmark (82)	Population sample adults, 15-69 years	1990-91	567	1.1
Denmark (85)	School children 12-16 years old	1995/96	717	1.8
Denmark (82, 87)	Population sample adults, 18-41 years	Jan-Nov 1998	414	2.7
Denmark (88)	Population sample adults, 18-69 years	June 2006–May 2008	3460	1.6
Norway (91)	Population sample adults, 18-69 years. (Results reported in 2007)	1994 (92)	1236	1.8 (1.1–2.7)
Germany (80)	Subgroup of MONICA sample, age 25-74	1994/95	1141	11.4
USA (86)	Student nurses, females	1980	85	17.6*
Sweden (81)	Population sample adults, age 20-65 years reporting hand eczema	1983-84	1087	5.8*

Note: * Testing performed with Fragrance Mix I, containing 16% allergens; the currently used Fragrance Mix I contains 8% allergens (see above).

Table 4-10: Results from patch testing with other fragrance allergens in different population based groups. If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS ⁽⁵⁾.

Country (Ref.)	Population	Year(s)	Fragrance allergen	No. tested	% positive (95% CI) [§]
Thailand (93)	Convenience sample (via advertisement), age 18-55	Not given	Isoeugenol, <i>Evernia prunastri</i> , <i>Myroxylon pereirae</i> *	2545	Positive to at least one of three allergens: 2.5 (1.9–3.2) [§]
Germany (80)	Subgroup of MONICA sample, age 25-74	1994/95	<i>Myroxylon pereirae</i>	1141	2.4
Denmark (88)	Population sample, age 18-69	1990 2006	<i>Myroxylon pereirae</i>	567 3460	1.1 0.1

Note: * *Myroxylon pereirae* is a balm obtained from a Central American tree. It is used as a screening substance for fragrance allergy in Europe and other geographical areas. Although the crude balm is not used in Europe in cosmetics, extracts and distillates are used (75).

4.4. Consumer products as a cause of fragrance contact sensitisation and allergic contact dermatitis

4.4.1. Clinical relevance

Clinical relevance is a concept used to describe the significance of a positive (allergic) patch test reaction for an individual patient: a reaction is deemed relevant if contact allergy to the substance is associated with previous or current episodes of allergic contact dermatitis. Thereby, the evaluation of clinical relevance links past exposure to morbidity. For the evaluation of relevance, past or recent exposure(s) to the allergen need to be identified in the patient's history. The success of this process generally depends on:

- The patient's understanding and awareness;
- The dermatologist's knowledge concerning exposures;
- Ingredient labelling; and
- Information about the actual chemical composition of the implicated product.

As these requirements may be met to a varying extent, the validity of relevance information as reported in clinical studies may also be variable. However, information on clinical relevance is important, in principle, because the proportion of currently relevant sensitisations reflects the amount of current exposure and resulting disease state, which may increase or decrease with time. In this way, current relevance also reflects the direct burden of a fragrance contact allergy to the individual and indirectly to society. Further important aspects of the evaluation of clinical relevance as a final step of patch testing have been discussed (32, 94-96).

Generally, clinical relevance is categorised as "current", "previous" or "unknown". Further differentiation has been introduced by adding information on:

- Occupational versus non-occupational causation; and
- The level of certainty of the relevance statement, e.g. as "certain", "probable", "possible".

In some cases, clinical relevance may not be established due to:

- Immunological cross-reactivity with an individual allergen, diagnosed or not;
- Active sensitisation by the patch testing;
- Contact sensitisation not caused by the substance, but by a contaminating constituent; or
- Failure to test with a true hapten (e.g. haptens formed from prehaptens on exposure to air, see chapter 5).

It should be noted that this statement on clinical relevance refers to the past history of a patient. This implies that a lack of, or unknown, clinical relevance does not make future allergen avoidance unnecessary.

In the context of contact allergy to fragrance ingredients, a number of alternative concepts of relevance have been used, for example:

- A history of intolerance to perfume or to perfumed products;
- A history of intolerance to perfume actually containing the allergen diagnosed;
- Detection of the culprit allergen in a perfume previously used.

4.4.2. Elicitation with clinical symptoms/signs, current and past

In case reports or small series, the clinical relevance of positive patch test reactions is usually well established and presented in detail. Moreover, a few large-scale clinical studies on contact allergy to fragrance ingredients have reported results on clinical relevance, which will be presented and discussed in this section. The studies can be subdivided into those which focus on medical history, patch testing with consumer products or detection of specific allergens in consumer products used by patients.

Medical history

A series of studies conducted in the 1990s showed that most individuals with contact allergy to fragrance ingredients were aware that they could not tolerate fragranced products on their skin and were able to specifically name product categories that initiated their disease (9). In this context, colognes, deodorants and lotions were named significantly more often by fragrance allergic dermatitis patients than by patients without fragrance contact allergy (3). These studies are described in the SCCNFP opinion on fragrance allergy of 1999 (1). Newer studies are outlined below.

NACDG 2009 study (65)

The definition of “present” clinical relevance in this North American network study was strict, requiring:

- A positive use or patch test with the suspected item(s) for “definite” relevance; and
- Verification of the presence of the allergen in known skin contactants, and consistent clinical presentation for “probable”.

If these conditions were not met, but skin contact to items generally containing the item was likely, “possible” was used.

Regarding fragrance allergens, the proportions were as described in Table 4-11.

Table 4-11: Extract from ((65) Table 3) regarding the proportion of patients with “present clinical relevance” (see text) and “past clinical relevance” (criteria not given).

Fragrance allergen	n (tested)	% (pos.)	Current relevance (%)			Past relevance (%)
			Definite	Probable	Possible	
<i>Myroxylon pereirae</i>	4449	11.9	1.3	33	53	2.7
FM I	4439	11.5	2.0	29.4	54.3	4.3
Cinnamal	4435	3.1	1.5	33.8	50	2.9
Ylang-Ylang oil	4434	1.5	4.6	10.8	73.8	1.5
Jasmine absolute	4447	1.1	0	24.5	67.3	6.1

Frosch 2002 (a) study (67)

In this study, 1,855 consecutive patients were patch tested with FM I and a series of a further 14 fragrance chemicals. Prior to the test, the history of adverse reactions to fragrances was classified as “certain” (6.6%), “probable” (8.0%), “questionable” (9.2%) or “none” (76.1%) (see (71)).

Frosch 2002 (b) study (97)

A series of 18 essential oils or components thereof, together with FM I, was assessed in 1,606 consecutive patients. Similar to the above study, the proportions of patients with a “certain” or “probable” history (or otherwise) and positive reactions to either FM I or the

special series, or both, were cross-tabulated. Of note, 53.7% of patients with positive reactions to FM I only, had no history. Similarly 54.2% of patients with positive reactions only to one of the essential oils had no history. However, in cases of reactivity to both FM I and one of the essential oils, the proportion of patients with no history was only 36.5%.

Frosch 2005 study (35)

The diagnostic properties of FM I and the new FM II were evaluated in 1,701 consecutive patients patch tested in six European centres. Contrasting a “certain” (found in 8.7% of patients) with “no history” (75.3% of patients), the sensitivity of FM I was 25.2%, and the positive predictive value (PPV) 45.1%. In comparison, the sensitivity of FM II at 14% concentration was 13.5% and the PPV was 55.6%. The combination of the two mixes was important, as more patients with a “certain” history, but also independently from history, reacted to just one of the mixes rather than to both.

Danish Contact Dermatitis Group 2005-2008 (69)

In 12302 consecutive patients patch tested in seven dermatology clinics and three university hospitals, 10.6% were positive to one or more of the fragrance allergy markers (FM I, FM II, *Myroxylon pereirae* or hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)). Clinical relevance covered current and/or past relevance based on: 1) medical history; 2) results of patch and/or use tests; 3) ingredient labelling; or 4) chemical analysis. Clinical relevance was found in 71.0% of cases positive to FM I, 72.2% of those positive to FM II and 76.7% of those positive to HICC. These proportions were higher than the average for other cosmetic allergens such as preservatives and hair dyes, which gave relevant reactions in about 50% of those positive, as did *Myroxylon pereirae*. *Myroxylon pereirae* itself is not used in cosmetics as it is banned, but sensitisation may be caused by exposures to related substances and thus relevance may be difficult to determine.

Cosmetic products

Fragrance formulae from cosmetic products

Popular fine fragrances (5), as well as toilet soaps, shampoos, lotions, deodorants, and aftershaves have been shown to provoke allergic contact dermatitis in patients when used for patch testing (5, 6, 98, 99). Moreover, commercially available fragrance formulae and dilutions of individual fragrance allergens were potent elicitors of allergic contact dermatitis under simulated use conditions (10, 100, 101).

More recently, deodorants spiked with the fragrance allergens cinnamal, hydroxycitronellal and HICC, respectively, in realistic in-use concentrations were shown to elicit allergic contact dermatitis in 89-100% of the fragrance allergic individuals tested (102-104). In 87.5% of HICC sensitised individuals the use of a cream (and in 82.8% the use of an ethanol solution) spiked with HICC provoked dermatitis (105). These studies are discussed in more detail in chapter 11 on quantitative aspects. Other new studies are mentioned below:

IVDK “own perfumes” study (106)

A different perspective on clinical relevance is provided by assessing the proportion of positive reactions to the FM I or single fragrance allergens in patients who had not tolerated certain perfumed products, such as deodorants and aftershaves and who were patch test positive to these cosmetics. The following two tables are taken from this publication.

Table 4-12: Extract from ((106) Table 2) on the frequency of positive reactions to fragrance allergens in patients with vs. without positive patch test reaction to their own deodorant.

Fragrance allergen	Conc. (%)	Deodorant positive (n=66)		Deodorant negative (n=855)	
		n (test)	% pos. (95% CI)	n (test)	% pos. (95% CI)
Fragrance Mix I	8	61	38.0 (24.1-51.9)	805	15.0 (12.5-17.5)
<i>Myroxylon pereirae</i>	25	60	22.9 (12.7-33.1)	806	9.1 (7.2-11.0)
Hydroxycitronellal	1	33	6.5 (0.7-12.3)	204	4.3 (1.5-7.1)
Isoeugenol	1	33	6.5 (0.7-12.3)	204	7.2 (3.6-10.8)
Cinnamal	1	29	11.3 (0-24.1)	133	1.1 (0-2.7)
Geraniol	1	29	8.3 (0-20.4)	141	0 (0-2.1)

Of the 66 patients with a positive patch test reaction to their own deodorant, most had positive reactions to one or more fragrance allergens. This was much more prevalent than those patients in whom no positive reaction to their deodorant was observed. This observation supports the notion that the respective fragrance allergens are important in contact allergy to fragrance ingredients caused by deodorants, supporting data regarding exposure (chapter 10.1).

Table 4-13: Extract from ((106) Table 2) on the frequency of positive reactions to fragrance allergens in patients with vs. without positive patch test reaction to their own aftershave, eau de toilette or perfume.

Fragrance allergen	Conc. (%)	Product positive (n=63)		Product negative (n=819)	
		n (test)	% pos. (95% CI)	n (test)	% pos. (95% CI)
Fragrance Mix I	8	56	57.1 (46.2-68.1)	764	13.9 (11.4-16.4)
<i>Myroxylon pereirae</i>	25	56	13.9 (7.3-20.4)	766	8.8 (6.8-10.7)
HICC	5	20	58.3 (37.5-79.0)	310	1.3 (0-2.7)
<i>Evernia prunastri</i>	1	28	22.1 (7.0-37.2)	153	8.8 (4.2-13.4)
Hydroxycitronellal	1	33	6.5 (0.7-12.3)	204	4.3 (1.5-7.1)
<i>Cananga odorata</i> (ylang-ylang oil)	10	7	16.3 (2.0-30.5)	43	5.0 (0-11.3)

Similar results were obtained from the subgroup of patients with a positive reaction to their eau de toilette, aftershave (hydroalcohol solutions) or perfumes (Table 4-13). However, notable differences were: (i) the greater relative importance of *Evernia prunastri* (Oak moss absolute); and (ii) generally an extremely high proportion of positive reactions to various other fragrance ingredients.

4.4.3. Elicitation in diagnostic patch tests without clinical history

In a variable proportion of patients, a positive patch test reaction does not correlate with recent or past episodes of presumptive allergic contact dermatitis. Apart from particular circumstances, such as cross-reactivity or reactivity to contaminants outlined above, there are several possible explanations for this:

- The patch test reaction was a false-positive (irritant).
- There was erroneous recall/interpretation of the patient's history (false-negative).
- Lack of knowledge concerning exposures.

- If the patient is weakly sensitised (e.g. by a low induction dose), the occlusive exposure during patch testing may have been the only exposure above the individual elicitation threshold capable of eliciting an unequivocal allergic contact reaction. In this situation, clinical relevance would be classified as “unknown”. Nevertheless, there is an alteration of the immune status of the individual.

Sometimes, a repeated open application or provocative use test is employed to mimic “normal” exposure to the allergen. A positive reaction to such a use-related test confirms actual sensitisation. Moreover, the positive result supports the necessity of future allergen avoidance. Apart from the risk of developing allergic contact dermatitis in the future, sensitisation means an alteration of the immune status of the individual.

4.5. Socio-economic impact of contact allergy

4.5.1. Health related quality of life

Skin diseases in general are known to affect quality of life significantly (107); this also applies to eczema, where most studies concern atopic dermatitis and hand eczema patients (108, 109). Hand eczema has a poor prognosis and may affect the self-image, limit social activities and lead to occupational restrictions (109, 110). The quality of life in hand eczema patients with fragrance contact allergy is affected in a similar degree as patients with other contact allergies (111).

In a questionnaire study of 117 patients recently diagnosed with contact allergy to fragrance ingredients, most presented with hand or facial eczema. In response to the question if and how fragrance allergy had affected their life situation, 67.5% replied that they often had to take special precautions, 47.0% replied that they were often bothered by eczema and itch, 17.1% said that they had had to take sick leave due to their fragrance contact allergy and 45.3% felt that fragrance contact allergy had significantly influenced their daily living (112).

4.5.2. Occupational restrictions

Contact allergy is known to influence severity and prognosis of hand eczema (113, 114) including risk of sick leave (111). Fragrance contact allergy is mostly of a non-occupational origin (90) related to the personal use of scented cosmetics, but may have secondary occupational consequences. This may be due to exposure to fragrance ingredients also in the work place or because hand eczema has developed. Hand eczema itself may make it impossible to remain in the trade even if protective equipment is used. In young people, fragrance allergy may limit the choice of occupations, as it will be difficult to work as a hairdresser, cosmetologist or in other occupations with a significant skin exposure to fragranced products.

4.5.3. Costs to health care/health economics

In a population based study of 3,460 individuals, contact allergy to FM I was found in 1.6%; logistic regression analyses showed that medical consultation due to cosmetic dermatitis (OR 3.37, 95% CI 1.83-6.20) and cosmetic dermatitis within the past 12 months (OR 3.53, CI 2.02-6.17) were significantly associated with sensitisation to FM I (88). Further, as mentioned above, fragrance allergy may lead to sick leave (112). No specific cost estimates for fragrance allergy exist, but the yearly total costs of contact dermatitis in Western Europe was estimated to be 5.2 billion Euro in 1997. Prices were based on the Allergy White Paper (1997) and on results of investigations and extrapolations of known data for Western Europe (115). Fragrance allergy is the second most frequent cause of contact allergy after nickel allergy and is seen in every 10th patient investigated for contact allergy. Even a modest reduction in nickel allergy has been estimated to have the value of 12 million Euro/year/million people in Denmark (Environmental Project Nr. 929, 2004; <http://www2.mst.dk/Udgiv/publications/2004/87-7614-295-7/pdf/87-7614-296-5.pdf>, last

accessed 2011-11-13). The costs are likely to differ in other countries, some with higher expenses and some with lower costs. These estimates show that the cost of contact allergy in the population may be considerable.

4.6. Allergen avoidance

Generally, “allergen avoidance” can be regarded as having two aspects: (i) primary prevention of the acquisition of contact allergy achieved by avoiding or limiting exposure of the general population, or certain parts of it, to allergens; and (ii) secondary prevention in terms of avoiding (re-)elicitation of allergic contact dermatitis in sensitised individuals.

4.6.1. Primary prevention: limiting or eliminating exposure to allergens in the population

The main aim of public health is the primary prevention of disease in populations. Allergic contact dermatitis (to fragrances) has the potential to have a significant impact on quality of life, including effects on fitness for work (chapter 4.5). Moreover, it is a common phenomenon and therefore a reduction of exposure to (fragrance) allergens must be an objective of effective Public Health measures.

Means of limiting or eliminating exposure to fragrance allergens include the following:

- *Prohibition* by regulatory measures or other means.
- *Restriction* by regulatory measures or other means of the maximum permissible concentration of a substance, or a critical component of natural mixtures, possibly according to different uses and product types, respectively.
- *Substitution* with suitable, but less or non-allergenic compounds. Substitution by a component which is chemically different, but effectively not different in terms of allergenicity or cross-reactivity, is not adequate (e.g. an ester) (chapter 5).
- *Formulating the fragrance* with the aim of limiting or eliminating those substances for which a sensitising potential has been shown. One difficulty with this approach is that sometimes no sensitisation data exist for those components of a fragrance formula which are used to replace a “known sensitiser”.
- *Deliberate avoidance* of the use of fragrances where they are not essential to the function of a finished product, but used merely to add to its appeal. Examples could include most cosmetics, topical medicaments, detergents etc., but obviously not perfumes, eau de toilette and other products used for their scent.
- *Information, e.g. labelling* so that the consumer may make an informed choice to avoid exposure to a particular ingredient.

4.6.2. Secondary prevention: avoiding re-exposure to (a) specific sensitiser(s) in clinically diagnosed individuals

In clinical dermatology, avoidance of re-exposure to an allergen is central to the care of sensitised patients. Contact sensitisation, as a latent condition, persists life-long, and therefore allergen avoidance is the only means of avoiding potentially severe and/or handicapping disease, which affects quality of life and may affect fitness for work, i.e. allergic contact dermatitis.

In this context, the valid diagnosis of sensitisation, by patch testing (32) with standardised materials, is a prerequisite of successful allergen avoidance.

In the case of fragrances, a history clearly indicative of “fragrance dermatitis” but in which patch testing with commercially available test preparations is negative, most probably reflects a shortcoming of the patch test procedure, namely, a false-negative investigation. An important cause is inadequate information on the presence of fragrance substances

present in cosmetic products (and consumer products in general). This means that patients cannot be tested for relevant substances.

A false-negative investigation can also be due to a number of other reasons: (i) non-adherence to scientific recommendations (32) or guidelines (e.g. (116)); (ii) sub-optimal patch test concentration; or (iii) use of non-oxidised material if oxidised material is the true allergen.

In an "ideal" case, from the point of view of successful patient management, the test procedure identifies all the allergen(s) to which the patient has developed contact allergy, according to the information on the culprit product(s) brought in by the patient. Such contact sensitisation is termed "clinically relevant" (65), and the need for allergen avoidance in the future is unequivocally evident in these cases. However, not infrequently, clinical relevance of an allergic patch test reaction cannot be ascertained for various reasons, which may be beyond control by the clinician (see chapter 4.4). Nevertheless, future elicitation of allergic contact dermatitis by sufficient contact with the identified "non-relevant" allergen may be expected. Hence, the patient will need to avoid the respective substance(s).

In a less "ideal" case, only part of the fragrance allergens having caused allergic contact dermatitis are identified (and can subsequently be avoided), while another part remains unidentified, for instance because it is: (i) not labelled on the product; and/or (ii) not available for routine diagnostic patch testing (special investigations such as chemical analysis of the culprit product, and break-down patch testing of its individual components, are performed rarely). Such "residual" undetermined sensitisation will hamper the success of secondary prevention of allergic contact dermatitis due to fragrances.

The above consideration raises the question for the patient of how to identify fragrance chemicals in cosmetics and other products coming into contact with the skin, such as detergents and household products, topical medicaments, products used professionally (e.g. by hairdressers, beauticians, masseurs, aromatherapists), and in other industrially used categories of products (7) (see also chapter 9). In this regard, the labelling with "perfume" or "contains fragrances" does not provide sufficient information. Moreover, such general labelling has two main disadvantages:

- It does not aid the identification of past exposure to specific agents when planning a patch test and later, when interpreting possible positive patch test results regarding clinical relevance.
- The diagnosis of allergic contact sensitisation to unidentified fragrance allergens will lead to unnecessary avoidance of other fragrance substances to which the patient is not sensitised, which are, however, included under the label "perfume".

Furthermore, the attribute "fragrance-free" may be misleading, as it merely states that no substance was added to the product to give it a scent, assuming it is used correctly at all. Nevertheless, fragrance substances used for other purposes, e.g. as preservatives, may expose the "fragrance allergic" patient to the allergen even in a "fragrance free" product (117). However, in terms of cosmetic ingredient labelling, such other uses are less problematic, as each ingredient not used as a fragrance component must be labelled. Also the use of natural products (essential oils) as preservatives must be considered in this context.

Ingredient labelling of 26 individual fragrance ingredients, identified as allergens in humans, was introduced for cosmetics in 2005. The intention was to provide a tool for clinicians for optimizing the investigation of patients with suspected fragrance allergy, as well as for fragrance allergic patients for avoiding products containing substances they have been shown to be allergic to. Both these aims are objectives of secondary prevention and seem to have been well accepted. In a study of fragrance allergic patients and their utilisation of ingredient labelling (112), most responded that they used the ingredient labelling (86.3%) and of those who used it, the majority (65.3%) found it helpful (112). Most allergic patients used the ingredient labelling (83.2%) to find out if the product was scented, while 35.6%

also looked for specific ingredients. Many (84.9%) found that a clearer labelling, e.g. easier names and a larger font size, would increase their benefit.

4.7. Conclusions

Contact allergy to fragrances is relatively common, affecting 1 to 3% of the general population, based on limited testing with eight common fragrance allergens and about 16 % of patients patch tested for suspected allergic contact dermatitis. Fragrance contact allergy is mostly non-occupational and related to the personal use of cosmetic products.

Allergic contact dermatitis can be severe and widespread, with a significant impairment of quality of life and potential consequences for fitness for work. Thus, prevention of contact sensitisation to fragrances, both in terms of primary prevention (avoiding sensitisation) and secondary prevention (avoiding relapses of allergic contact dermatitis in those already sensitised), is an important objective of public health risk management measures.

5. Activation of weak or non-sensitising substances into sensitisers - prehaptens and prohaptens

Fragrance allergens act as haptens, i.e. low molecular weight chemicals that are immunogenic only when attached to a carrier protein. However, not all sensitising fragrance chemicals are directly reactive, but require previous activation.

A prehapten is a chemical that itself is non- or low-sensitising, but that is transformed into a hapten outside the skin by simple chemical transformation (air oxidation, photoactivation) and without the requirement of specific enzymatic systems.

A prohapten is a chemical that itself is non- or low-sensitising but that is transformed into a hapten in the skin (bioactivation) usually via enzyme catalysis.

It is not always possible to know whether a particular allergen that is not directly reactive acts as a prehapten or as a prohapten, or both, because air oxidation and bioactivation can often give the same product (geraniol is an example).

Some chemicals might act by all three pathways. One example is geranial (an isomer of citral) which is a hapten itself with a moderate sensitisation potency, but can be activated to more potent sensitisers via air oxidation (autooxidation) thus acting as a prehapten and also via bioactivation (metabolic activation) thus acting as a prohapten (118).

Increased understanding of the importance of activation through interaction with the environment that turns non-sensitising compounds into sensitisers has made it important to distinguish between prehaptens and prohaptens. This distinction facilitates discussions by emphasizing the differences in activation mechanisms between the two types of compounds requiring activation to become haptens. It is important to note that prehapten activation, in contrast to bioactivation, can be prevented to a certain extent by avoidance of air exposure during the handling and storage of the chemicals. This concerns the most prominent haptens formed by autooxidation i.e. the hydroperoxides. In bioactivation, hydroperoxides have not been identified as metabolites, but other allergenic oxidation products (in particular aldehydes and epoxides) have been identified as being formed by both activation routes depending on the structure of the compound. One thoroughly studied example is geraniol which forms the aldehyde geranial, epoxy-geraniol, and also epoxy-geranial via both pathways of activation (autooxidation and metabolic oxidation) (119, 120). When haptens are formed by both pathways, the impact on the sensitisation potency depends on the degree of autooxidation in relation to the amount of metabolic oxidation.

Human data on established prehaptens are presented in Table 5-1 and Table 5-2. In Table 5-1 the results from patch testing with air exposed samples of the prehaptens are given. Table 5-2 shows the results from testing with the prehaptens themselves without intended air exposure. In addition to the data given in this chapter, animal data (LLNA) on the pure prehaptens or after controlled air exposure are given in Table 8-2. Possible pro- and prehaptens are identified by SAR analyses in chapter 9.

5.1. Prehaptens

Autooxidation is a free radical chain reaction in which hydrogen atom abstraction in combination with addition of oxygen forms peroxy radicals. The reaction shows selectivity for positions where stable radicals can be formed. So far, all fragrance substances that have been investigated with regard to the influence of autooxidation on the allergenic potential, including identification of formed oxidation products, have oxidisable allylic positions that are able to form hydroperoxides and/or hydrogen peroxide as primary oxidation products upon air exposure. Once the hydroperoxides have been formed outside the skin they form specific antigens and act as skin sensitisers (121). Secondary oxidation products such as aldehydes and epoxides can also be allergenic, thus further increasing the sensitisation potency of the autooxidation mixture (122). The process of photoactivation may also play a role, but further research is required to establish whether this activation route is currently underestimated in importance due to insufficient knowledge of the true haptens in this context.

Most terpenes with oxidisable allylic positions can be expected to autoxidise on air exposure due to their inherent properties. Depending on the stability of the oxidation products that are formed, a difference in the sensitisation potency of the oxidised terpenes can be seen. Oxidation products of commonly used fragrance terpenes (limonene, linalool, geraniol, linalyl acetate) have been identified as potent sensitisers in predictive animal tests (119, 123-128) (see chapter 8). This is also demonstrated for alpha-terpinene (129) and citronellol (AT Karlberg, personal communication 2012). The oxidised fragrance terpenes limonene, linalool and linalyl acetate have been tested in consecutive dermatitis patients and give frequent allergic contact reactions (130-135). Not all oxidised fragrance substances are strong sensitisers, e.g. caryophyllene is readily oxidised but has a low sensitisation potency after autoxidation (136). This is supported by clinical studies showing oxidised caryophyllene to be a less frequent allergen compared to oxidised limonene and oxidised linalool (133). Details are given in Table 5-1 The non-oxidised compounds rarely cause allergic reactions (43-45, 67, 70, 74, 97, 137-139), for details see Table 5-2. As oxidised and non-oxidised fragrance terpenes were not patch tested simultaneously in the same patients, the results are presented in two separate tables (Table 5-1 and Table 5-2).

Oxidised fragrance terpenes with defined content of the major haptens formed after autoxidation have not been commercially available for testing in dermatology clinics. In the published clinical studies testing oxidised fragrance terpenes, the patch test preparations have been obtained specifically for the performed multicentre studies. From 2012, patch test preparations of oxidised limonene and oxidised linalool with defined content of the major allergens in the oxidation mixtures, i.e. the hydroperoxides, are commercially available.

It should be noted that activation of substances via air oxidation results in various haptens that might be the same or cross-reacting with other haptens (allergens). The main allergens after air oxidation of linalool and linalyl acetate are the hydroperoxides. If linalyl acetate is chemically hydrolysed outside the skin it can thereafter be oxidised to the same haptens as seen for linalool. A corresponding example is citronellol and citronellyl acetate. In clinical studies, concomitant reactions to oxidised linalool and oxidised linalyl acetate have been observed (140, 141). Whether these reactions depend on cross-reactivity or are due to exposure to both fragrance substances cannot be elucidated as both have an allergenic effect themselves.

Linalool and linalyl acetate are the main components of lavender oil. They autoxidise on air exposure also when present in the essential oil, and form the same oxidation products found in previous studies of the pure synthetic terpenes. Experimental sensitisation studies showed that air exposure of lavender oil increased the sensitisation potency. Patch test results in dermatitis patients showed a connection between positive reactions to oxidised linalool, linalyl acetate and lavender oil (140).

Air oxidation of prehaptenes can be prevented to a certain extent by measures during handling and storage of the ingredients and final products to avoid air exposure, and/or by addition of suitable antioxidants. The autoxidation rate depends not only on the compound itself, but also on its purity. The prevention of autoxidation using antioxidants needs thorough investigation because antioxidants can exert their function by being oxidised instead of the compound that they protect and might thereby be activated to skin sensitising derivatives after oxidation, which is the case for alpha-terpinene from tea tree oil (129). Alpha-Terpinene together with its analogue gamma-terpinene has been suggested as an agent for maintaining the oxidative stability of different matrices, such as food, cosmetics and medicaments (142-144). As antioxidants are now frequently used at elevated concentrations in scented products due to a growing awareness of the problem of autoxidation, there is a risk that sensitisation caused by the antioxidants will rise. One of the most used antioxidants is butylated hydroxytoluene (BHT) which is considered a minimal risk for sensitisation in the concentrations used but nevertheless, with increased concentrations and usage, the risk of sensitisation could increase.

Due to the complexity of scented products, which are mixtures of many different fragrance substances, there are at present no published data identifying the presence of individual hydroperoxides in cosmetic products containing the above fragrance terpenes. However, clinical studies show a clear connection between contact allergy to oxidised limonene and oxidised linalool, and contact allergy to other markers of fragrance contact allergy (130-135); see Table 5-3.

Table 5-1: Contact allergic reactions to the autoxidised fragrance substances limonene, linalool, caryophyllene, myrcene and linalyl acetate in consecutive dermatitis patients.

INCI name	CAS no	Test conc. (%)	n Positive/n tested (%)	Comments (Ref.)
D-Limonene (ox.)	5989-27-5	5	18/703 (2.6%)	§ (130)
		3	28/1172 (1.6%)	
		2	3/362 (0.83%)	
D-Limonene (ox.)	5989-27-5	3	63/2273 (2.8%) variation between centres: 0.3-6.5%	§ (131)
D-Limonene (ox.)	5989-27-5, 5989-54-8, 138-86-3	3	49/1812 (2.3%)	§ (134)
L-Limonene (ox.)			36/1812 (2.0%)	
D – and/or L- Limonene (ox.)			63/2411 (2.6%)	
Linalool (ox.)	78-70-6	2	20/1511 (1.3%) variation between centres: 0.4-2.7%	§ (133)
Caryophyllene (ox.)	88-44-5	3.9	2/1511 (0.1%)	
Myrcene (ox.)	123-35-3	3	1/1511 (0.1%)	
Linalool (ox.)	78-70-6	2	14/1693 (0.83%)	§ (135)
		4	67/2075 (3.2%)	
		6	91/1725 (5.3%)	
		11	72/1004 (7.2%)	
Linalool (ox.)	78-70-6	3	11/483 (2.3%)	(145)
Linalyl acetate (ox.)	115-95-7	6	13/1217 (1.1%)	(141)

Notes: § Bicentric or multicentre studies.
(ox.) Oxidised.

Table 5-2: Contact allergic reactions to limonene, linalool, linalyl acetate and caryophyllene in consecutive dermatitis patient. Please observe that several studies have been performed using the test substances without reporting the autoxidation status but it has been intended to be low. For precise information see the original references.

INCI name	CAS number	Test conc. (%)	n Positive/n tested (%)	Comments (Ref.)
Limonene	138-86-3	2	0/1200	(137)
Limonene			3/2396 (0.1%)	§ (74)
DL-Limonene			11/1241 (0.88%)	§ (43)
Limonene			0/320	(44)
DL-Limonene			3/2396 (0.1%)	§ (74)
Linalool	78-70-6	30	0/179	(139)
		20	3/1825 (0.2%)	§ (45)
		10	2/320 (0.6%)	(44)
		10	4/792 (0.5%)	(138)
		5 and 1	0/100	(70)
		10	7/2401 (0.3%)	§ (74)
Linalool, "stabilised" *		10	2/985 (0.2%)	§ (43)
Linalyl acetate	115-95-7	1, 5	0/100	(70)
		10	4/1855 (0.2%)	§ (67)
beta-Caryophyllene	87-44-5	5	10/1606 (0.6%)	§ (97)

Notes: § Bicentric or multicentre studies.

(ox.) Oxidised.

* Stabilised: according to the manufacturer contained additional substances aimed at limiting oxidation.

Table 5-3: Concomitant reactions to fragrance markers: Fragrance Mix I and II (FM I, FM II), *Myroxylon pereire* (MP) and to colophonium (coloph.) in the baseline series in patients with positive or negative patch test reactions to oxidised fragrance substances.

	Total number of pos. and/or neg. reactions	Pos. to FM I		Pos. to MP		Pos. to coloph.		Ref.
		n	%	n	%	n	%	
Reactions to ox. D- limonene and/or limonene hydroperoxide fraction	Pos.: 49	20	41	12	24	12	24	(130)*
	Neg.: 2751	223	8.1	142	5.2	131	4.8	
Reactions to ox. D- limonene and/or limonene hydroperoxide fraction ^a	Pos.: 60	22	37	11	18	13	22	(132)*
	Neg.: 729	141	19	71	9.7	58	8	

Reactions to ox. D- limonene and/or ox. L- limonene ^a	Pos. to ox. D- limonene: 41	14	34	11	27	11	27	(134)*		
	Neg. to ox. D- limonene: 1771	113	6.4	91	5.1	62	3.5			
	Pos. to ox. L- limonene: 36	11	31	12	33	9	25			
	Neg. to ox. L- limonene: 1776	116	6.5	80	4.5	64	3.6			
Reactions to any of ox. linalool, myrcene, caryophyllene	Pos. to any of the tested ox. subst.: 31	12	39	6	31	12	39	(133)*		
	Neg. to any of the tested ox. subst: 1480	93	6	63	4	46	3			
		Pos. to FM I		Pos. to FM II		Pos. to MP		Pos. to coloph.		
		n	%	n	%	n	%	n	%	
Reactions to ox. linalool	Pos. at test conc. 4%: 30	8	26.7	5	16.7	10	33.3	5	16.7	(135)*
	Pos. at test conc. 6%: 55	12	21.8	8	14.5	11	20	8	14.5	
	Pos. at test conc. 11%: 72	14	19.4	9	12.5	14	19.4	9	12.5	
	Total pos. at any test conc: 75/1004	n.g.		n.g.		n.g		n.g.		
	Total neg. at any test conc: 929/1004	56	6.0	29	3.1	45	4.8	24	2.6	

Notes: * Bicentric or multicentre studies.

n.g. Not given.

(ox.) Oxidised.

5.2. Prohaptens

Compounds that are bioactivated in the skin and thereby form haptens are referred to as prohaptens. The human skin expresses enzyme systems that are able to metabolise xenobiotics (146), modifying their chemical structure to increase hydrophilicity and allow elimination from the body. Xenobiotic metabolism can be divided into two phases: phase I and phase II. Phase I transformations are known as activation or functionalisation reactions, which normally introduce or unmask hydrophilic functional groups. If the metabolites are sufficiently polar at this point they will be eliminated. However, many phase I products have to undergo subsequent phase II transformations, i.e. conjugation to make them sufficiently water soluble to be eliminated. Although the purpose of xenobiotic metabolism is detoxification, it can also convert relatively harmless compounds into reactive species. Cutaneous enzymes that catalyse phase I transformations include the cytochrome P450 mixed-function oxidase system, alcohol and aldehyde dehydrogenases, monoamine oxidases, flavin-containing monooxygenases and hydrolytic enzymes. Acyltransferases, glutathione S-transferases, UDP-glucuronosyltransferases and sulfotransferases are

examples of phase II enzymes that have been shown to be present in human skin (146). These enzymes are known to catalyse both activating and deactivating biotransformations (147), but the influence of the reactions on the allergenic activity of skin sensitisers has not been studied in detail.

Skin sensitising prohaptens can be recognised and grouped into chemical classes based on knowledge of xenobiotic bioactivation reactions, clinical observations and/or *in vivo* and *in vitro* studies of sensitisation potential and chemical reactivity. Few mechanistic investigations of prohaptens have so far been published. Investigations that are important for the bioactivation of fragrance substances are studies on alkenes, e.g. alpha- terpinene (148-150), the allylic primary alcohols geraniol (120) cinnamyl alcohol (151-155), eugenol and isoeugenol (156).

In order to be able to predict the sensitisation potency of prohaptens, steps of bioactivation have to be included in the predictive tests where intrinsic bioactivating systems are lacking. So far, no such predictive non-animal methods have been developed that take account of this.

When bioactivation occurs, the risk of cross-reactivity also needs to be considered. Cross-reactivity between certain aldehydes and their corresponding alcohols, e.g. cinnamal - cinnamyl alcohol and geranial - geraniol, due to the metabolic oxidation of the alcohols to the aldehydes in the skin is demonstrated (120, 151-155).

When using derivatives of a fragrance substance, it must be taken into account that the derivative could be metabolically transformed in the skin into the parent or cross-reacting compounds. A prominent example of such bioactivation is the hydrolysis of esters by esterases to the corresponding original alcohols. The metabolic product obtained can act as a hapten or a prohaptens in exactly the same way as the non-esterified parent compound.

Isoeugenol and its derivatives are an important example for this mechanism from which general conclusions may be drawn. As only the use of isoeugenol in fragranced products needs to be indicated on the ingredients list, the additional exposure to isoeugenol through its derivatives should also be taken into account. In a study it was shown that several EDP/EDT/aftershave lotions contained high levels of isoeugenyl acetate and isoeugenol methyl ether (Table 5-4) (157). Isoeugenyl acetate will be hydrolysed by esterases in the skin to generate isoeugenol. The situation may be similar for eugenyl acetate and geranyl acetate, which might be used in fragrance formulations instead of eugenol and geraniol, respectively. Moreover, such derivatives will contribute to exceeding any established 'acceptable dose/area level' of the parent compound, i.e., yield unduly high concentrations on the skin.

Table 5-4: Mean and median content of isoeugenol and its derivatives in the 29 perfume products.

Fragrance compound INCI Name	Products containing the fragrance		Content (ppm)			
	No.	%	Range	Mean	SD	Median
Isoeugenol	16	55	27-203	71	54	45
Isoeugenyl acetate	10	34	20-4689	985	1570	166
Isoeugenyl methyl ether	13	45	65-1755	360	442.3	222

5.3. Conclusions

- Many fragrance substances can act as prehaptenes or prohaptenes, forming allergens which are more potent than the parent substance by abiotic and/or metabolic activation. Activation can thus increase the risk of sensitisation.
- Fragrance substances of clinical importance known to be prehaptenes and to form sensitising compounds by air oxidation include limonene, linalool, and linalyl acetate.
- Fragrance substances of clinical importance known to be prohaptenes and to form sensitising compounds by metabolic transformation include cinnamyl alcohol, eugenol, isoeugenol and isoeugenol acetate.
- Fragrance substances of clinical importance with published data known to be both prehaptenes and prohaptenes and to form sensitising compounds by air oxidation (prehaptenes) and by metabolic transformation include geraniol and alpha-terpinene.
- A fragrance substance that sensitises without activation, but forms more potent sensitising compounds by air oxidation and also by metabolic transformation is, as one example, geranial (one isomer of citral).
- In the case of prehaptenes, it is possible to prevent activation outside the body to a certain extent by different measures, e.g. prevention of air exposure during handling and storage of the ingredients and the final product, and by the addition of suitable antioxidants. When antioxidants are used, care should be taken that they will not be activated themselves and thereby form new sensitisers.

It should be noted that the possibility to reduce the sensitisation potency by preventing air oxidation is also important for a direct acting hapten or prohapten, if a further activation by air oxidation to more allergenic compounds has been shown.

- In the case of prohaptenes, the possibility to become activated is inherent to the molecule and activation cannot be avoided by extrinsic measures. Activation processes increase the risk for cross-reactivity between fragrance substances. Cross-reactivity has been shown for certain alcohols and their corresponding aldehydes, i.e. between geraniol and geranial (citral) and between cinnamyl alcohol and cinnamal.

Cross-reactivity is also expected between ester derivatives and their parent alcohols, as the esters will be hydrolysed by esterases in the skin. Esters of important contact allergens that can be activated by hydrolysis in the skin are isoeugenol acetate, eugenyl acetate and geranyl acetate all of which are known to be used as fragrance ingredients.

- Further experimental and clinical research in the area of abiotic and/or metabolic activation of fragrance substances is clearly needed to increase the safety for the consumer. Compounds suspected to act as prehaptenes and/or prohaptenes should be considered as allergens, unless it could be demonstrated that they do not become activated by one of the described pathways.

6. Retrieval of evidence and classification of fragrance substances

For a systematic review, a structured approach of identifying, grading and aggregating available information should be used. Regarding the classification of substances as allergens, a number of approaches have been suggested (158-160). The categorisation of skin sensitisers according to sensitising potency has also been proposed (161, 162). For this opinion, these discussions were extended to reconcile different perspectives and to arrive at a strategy that is both consistent and applicable in practice.

By default, positive human evidence (clinical data) overrides negative results obtained in animals. This implies that the observation of a sufficient number of positive clinical cases is more important than potency information derived from animal experiments (LLNA).

Cosmetovigilance information based on consumer complaints only is of limited value in the evaluation of sensitisation risk associated with cosmetic allergens, including fragrances, as it does not identify specific causative substances, and likely to severely under-estimate the frequency of contact dermatitis. An exception is the combination with qualified diagnostic work-up, as in the French REVIDAL/GERDA system (299); however, such data are generally published, thus publicly available, and considered in the present opinion.

6.1. Retrieval of evidence

A systematic search strategy was employed for the retrieval of clinical data, as outlined below. Experimental data are often not published hence the exact definition of the scope considered for the review is necessary and is given below. Additional LLNA data were reviewed, if identified by the search strategy, e.g. in chapter 8.1.2 and, as "additional information", in Annex I of this opinion. This supplemental evidence was, however, not considered for the final categorisation in Table 13-2.

6.1.1. Search strategy for clinical data

Method of literature search:

1. Manual search of the issues of the journal "Contact Dermatitis" (for the 26 "annex substances", which were re-evaluated in the present opinion, starting 1999) up to October 2010, identifying all studies with fragrance substances.
2. PubMed search of CAS numbers identified in the previous opinion, reviews and already identified clinical studies, respectively, and manual screening of identified publications (narrowed for the last 10 years for the 26 "annex substances"), if necessary narrowing the search results by adding "dermatitis" or "allergy". For example, for citral: 5392-40-5 AND (dermatitis or allergy), translated into
 "5392-40-5"[EC/RN Number] AND
 (
 ("dermatitis"[MeSH Terms] OR "dermatitis"[All Fields])
 OR
 ("hypersensitivity"[MeSH Terms] OR "hypersensitivity"[All Fields] OR "allergy"[All Fields] OR "allergy and immunology"[MeSH Terms] OR ("allergy"[All Fields] AND "immunology"[All Fields]) OR "allergy and immunology"[All Fields])
)
3. Manual search of all RIFM reviews published in supplement issues of "Food and Chemical Toxicology"² in the past 20 years. In case of the least evidence on human sensitisation the substances were preliminarily selected and further research initiated.

² Food and Chemical Toxicology, Elsevier Ltd. <http://www.sciencedirect.com/science/journal/02786915>.

4. Consideration of the most important ("top 100") fragrance compounds in terms of volumes used (disregarding functional additives such as solvents) as supplied by the International Fragrance Association IFRA (personal communication 2010).
5. Consideration of fragrance compounds ranking 101 to 200 on the list of use volumes, if they were self-classified by manufacturers as skin sensitisers (R 43).

For the present systematic overview of available clinical data, only original studies were considered, as only these provide direct evidence, while other reviews, partly being based on the same original reports, only served to identify additional literature. In contrast, selected reviews, guidelines and similar publications were used as basis for methodological approaches (e.g., in section 11).

6.1.2. Collection of experimental (LLNA) data

The SCCS requested the International Fragrance Association (IFRA) to submit data on animal tests performed with fragrance substances, to be presented in a structured format. In response, industry submitted first a poster (163) and later a report consisting of LLNA protocol summaries on the 59 fragrance substances in the poster (164). No guinea pig studies were submitted. The SCCS has reviewed and analysed the report and the publications quoted in the report. A summary is given in chapter 8 and full data are given in Annex II. EC3 values on some additional fragrance substances in two published reviews (165, 166) have also been considered. Additional EC3 values may be available in the scientific literature and there may also be other unpublished data.

6.2. Grading of evidence

Assembled evidence has to be graded in two steps: (i) the quality of each single study, and (ii) the strength of evidence underlying the eventual classification as an allergen. Generally, studies (published or not) which are eligible for consideration will contribute to the final overall judgement to different degrees.

- Positive human data, if sufficiently demonstrated (point (i) below), will always over rule experimental (animal), *in vitro* or *in silico* data of similar internal validity, as they provide direct evidence on allergenicity in humans.
- Small study groups will contribute less precise information than larger studies of otherwise similar quality. As a minimum requirement, the size of the study groups and the numbers of events must be stated in the reports.

The following subsections will address special aspects of clinical and experimental studies, respectively.

6.2.1. Quality of a clinical study

Two major types of clinical studies must be distinguished because they provide a different scope of information:

- (i) Case reports or small case series, focusing on patients with positive (test) reactions to the target substance, sometimes including a set of non-exposed, possibly non-diseased "control patients"; these should present a concise summary of all relevant aspects of the patient's history, diagnostic procedures and possibly further outcomes.
- (ii) Clinical series in which results of a group of patients patch tested with the target substance, often combined with other substances, are presented. In the latter type of report, usually only a minority of patients tested show a positive reaction to the test substance. This implies that the majority of patients can be used to illustrate the proportion of irritant, doubtful and negative reactions. The degree of detail on the patients' histories is usually limited in such studies, compared to case reports.

Some of the basic quality criteria in clinical patch testing which should be considered are:

- Adherence to international patch test guidelines (32, 96).
- Material(s) tested should be characterised.
- Total number of patients tested must be given.
- Patient selection should be described.
- Relevance may be demonstrated either on a case-by-case basis, following pertinent guidelines, or in terms of a significant epidemiological association between sensitisation and exposure or valid markers of exposure.

Concerning relevance, it must be noted that while clinical relevance can provide important information (see 4.4.1), it is ideally based on comprehensive knowledge of prior exposures. Since the implementation of labelling 26 fragrances, previous exposure to these can often be ascertained in the assessment of relevance of a positive patch test reaction (44). However, exposure to substances not listed on a product ingredient label is obscure, except in very rare cases where elaborate diagnostics and chemical analyses are feasible (e.g. (167)). Thus, a lack of information on relevance (reported in studies) does not invalidate the impact of diagnosed contact sensitisation.

6.2.2. Quality of an experimental study

International guidelines such as the pertinent OECD guidelines for testing sensitisation have been developed and adopted. Experimental studies following these guidelines are considered as valid. However, a vast number of non-guideline studies are available and should be assessed on a case-by-case basis.

6.2.3. Quality of “other” evidence

Supporting evidence besides human and animal (experimental) data comprises *in vitro* test systems, *in chemico* experiments and structure activity relationships (SARs).

SAR analysis has at present no formal regulatory validation for skin sensitisation, nevertheless it may provide useful indicative information on sensitising potential when no or limited clinical or animal data are available.

SAR studies must consider a possible formation of haptens (allergens) from compounds able to act as prehapten by, e.g. autoxidation outside the body as well as metabolic activation in the skin of compounds able to act as prohapten (122, 168).

6.3. Aggregating evidence for a final conclusion

The criteria listed below are followed as a flow chart to arrive at a conclusion. This implies that if classification into one category is achieved, subsequent categories need not be considered. Based on the above criteria, fragrance substances were selected to be included in the present opinion if classified in one of the categories defined below.

6.3.1. Established contact allergen in humans

To qualify as an *established contact allergen*, the SCCS considers that *at least one* of the following two criteria must be met:

- At least two clinical series fulfilling the quality criteria from two different centres with cases of sensitisation, or at least three separate clinical series from different centres if a study, or studies, do not meet all quality criteria. (→ *sufficient human evidence present*)
or
- Case reports from at least two independent centres describing more than two

patients altogether in whom clinically relevant contact sensitisation had unequivocally been proven (→ *sufficient human evidence present*)
or

- At least one clinical series fulfilling the quality criteria, together with at least one case report of clinically relevant contact sensitisation (→ *sufficient human evidence present*);
or
- Experimentally induced sensitisation (e.g. unequivocally positive human maximisation tests/repeated insult patch test)³ (→ *sufficient human evidence present*).

6.3.2. Established contact allergen in animals

To qualify as an *established contact allergen*, the following criterion must be met:

- At least one positive animal study carried out according to accepted guidelines, providing evidence of a sensitisation potential (→ *sufficient animal evidence present*).

6.3.3. Likely contact allergen, if human, animal and other evidence is considered

To qualify as an *likely contact allergen*, if classification as “established ...” is not applicable, *at least two* of the following criteria must be met:

- Individual cases of allergic patch test reactions not fulfilling the requirements for sufficient evidence (→ *limited human evidence present*)
or
- At least one positive non-guideline animal study, which should be evaluated on a case-by-case basis (→ *limited animal evidence present*)
or
- Other evidence, e.g. results from *in chemico* experiments or *in vitro* tests or from structure-activity considerations based on sufficiently valid results for closely related compounds (→ *other evidence present*).

6.3.4. Possible contact allergen, if human, animal and other evidence is considered

To qualify as a *possible contact allergen*, if classification as “established ...” or as “likely ...” contact allergen is not applicable, *at least one* of the following criteria must be met:

- Individual cases of allergic patch test reactions not fulfilling the requirements for sufficient evidence (→ *limited human evidence present*)
or
- At least one positive non-guideline animal study, which should be evaluated on a case-by-case basis (→ *limited animal evidence present*)
or
- Other evidence, e.g. results from *in chemico* experiments or *in vitro* tests or from structure-activity considerations based on sufficiently valid results for closely related compounds (→ *other evidence present*).

³ It should be noted that the SCCS considers such tests unethical (169. SCCP. Opinion concerning the predictive testing of potentially cutaneous sensitising cosmetic ingredients or mixtures of ingredients adopted by the SCCNFP during the 11th plenary session of 17 February 2000. 2000:).

6.4. Conclusions

The present opinion includes (i) a well-defined search strategy for retrieving pertinent evidence; (ii) a definition of criteria used to evaluate available evidence; and, finally (iii) a set of rules to categorise the substances with regard to the relevant toxicological endpoint, i.e. sensitisation in man, based on the evidence.

7. Reported fragrance allergens from the clinical perspective

In this chapter, clinical evidence regarding sensitisation to individual fragrance chemicals and to natural extracts (essential oils) is tabulated. In this report “single chemicals” refers to chemicals of natural or synthetic origin whose chemical identity is fully known. The term “natural extracts” refers to plant or animal derived mixtures of natural chemicals, for example lavender oil, whose composition may be variable and may or may not have been fully or partly established. Full information, including possible synonyms, structural formulas (in the case of single chemicals only), a short summary of available evidence and further information, e.g. on regulatory status, is presented in Annex I.

7.1. Tabular summary of evaluated individual fragrance chemicals

Regarding nomenclature, INCI names are used wherever possible. If an INCI name is not available, the perfuming name as listed by CosIng is used. Detailed information on the publications identified and considered for this report can be found in Annex I. Several substances are currently banned from the use in cosmetic products by Annex II of the Cosmetics Directive, based on concerns regarding one or more toxicological endpoints. While available clinical evidence regarding this set of substances is listed in Annex I, these substances have not been further evaluated and are thus not included in this chapter.

In this section, a tabular overview on the classification of substances considered is presented in four tables listing:

1. Established contact allergens in humans (→ *sufficient human evidence present*).
2. Substances with positive human data, which are, however, not sufficient to categorise as “established contact allergen in humans” (→ *limited human evidence present*).
3. Substances with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.
4. Substances eligible for inclusion (see beginning of chapter 6) for which no human data are available.

A critical point in understanding this scheme is that there is publication bias in reporting allergens. This is due to the fact that once a substance has been reported and accepted as a contact allergen in humans, further reports are less likely to be published unless they are part of an epidemiological survey or when there is a novel source of exposure. Moreover, the number of patients displaying positive test reactions obviously not only depends on the underlying prevalence of sensitisation, but also on how often a substance is patch tested. This implies that inclusion of an allergen or allergen mixture in the baseline patch test series (as for Fragrance Mix I and II, *Myroxylon pereirae* and HICC, and partly also other substances/mixtures) will yield the maximum possible number of cases. In contrast, patch testing in “special” series, e.g. as a break-down of single constituents of the respective mix in case of a positive reaction to the latter, or with application only in the case of strongly suspected fragrance intolerance, will mostly result in higher relative numbers than testing the same compound consecutively, but also in lower absolute numbers.

In Table 7-1, the single substances are listed with a semi-quantification of their impact which were categorised as established contact allergens in humans according to the criteria given in chapter 6.3.

Established contact allergens in humans, according to the criteria outlined in chapter 6.3.1, were categorised according to the number of patients reacting positively and to the number of patients tested, based on the publications considered (see annex I for references). The following categories were used:

+	Up to 10 positive test reactions reported
++	11 to 100
+++	101 to 1000
++++	> 1000

If a test allergen has been tested in less than 1,000 patients, "r.t." (rarely tested) is added in the following tables. For this categorisation, absolute numbers of cases of sensitisation, and not the relative frequency of positive patch tests, were used, because relative frequencies depend heavily on the selection of patients for patch testing. Thereby, an important allergen tested routinely, in the baseline series, may yield 1 to 2% positive reactions (usually in several thousand patients), while an allergen tested in a selective fashion (in much fewer patients) may yield an even higher relative frequency. Moreover, case reports/series cannot be interpreted in terms of relative frequencies. The calculation of absolute numbers was based on all available literature, as detailed in the annex I to this opinion, i.e., regarding the 26 substances already listed in Annex III to the Cosmetics Directive includes data already evaluated in the previous opinion.

Table 7-1: Established contact allergens in humans (summary of evaluation as detailed in chapter 6.3). More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment: see text
ACETYLCEDRENE	32388-55-9	+
AMYL CINNAMAL	122-40-7	++
AMYL CINNAMYL ALCOHOL	101-85-9	++
AMYL SALICYLATE	2050-08-0	+
trans-ANETHOLE	4180-23-8	+ (r.t.)
ANISYL ALCOHOL	105-13-5	+
BENZALDEHYDE	100-52-7	+
BENZYL ALCOHOL	100-51-6	++
BENZYL BENZOATE	120-51-4	++
BENZYL CINNAMATE	103-41-3	++
BENZYL SALICYLATE	118-58-1	++
BUTYLPHENYL METHYLPROPIONAL (Lilial®)	80-54-6	++
CAMPHOR	76-22-2 / 464-49-3	+ (r.t.)
beta-CARYOPHYLLENE (ox.)	87-44-5	Non-ox.: +, ox.: +
CARVONE	99-49-0 / 6485-40-1 / 2244-16-8	+ (r.t.)
CINNAMAL	104-55-2	+++
CINNAMYL ALCOHOL	104-54-1	+++
CITRAL	5392-40-5	+++
CITRONELLOL	106-22-9 / 1117-61-9 / 7540-51-4	++
COUMARIN	91-64-5	+++
(DAMASCENONE)	23696-85-7	+ (r.t.)

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment: see text
ROSE KETONE-4		
alpha-DAMASCONE (TMCHB) [#]	43052-87-5 / 23726-94-5	++
cis-beta-DAMASCONE [#]	23726-92-3	+
delta-DAMASCONE [#]	57378-68-4	+
DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	151-05-3	+
EUGENOL	97-53-0	+++
FARNESOL	4602-84-0	+++
GERANIOL	106-24-1	+++
HEXADECANOLACTONE	109-29-5	+ (r.t.)
HEXAMETHYLINDANOPYRAN	1222-05-5	++
HEXYL CINNAMAL	101-86-0	++
HYDROXYISOHEXYL 3-CYCLOHEXENE CARBOXALDEHYDE (HICC)	31906-04-4 / 51414-25-6	++++
HYDROXYCITRONELLAL	107-75-5	+++
ISOEUGENOL	97-54-1	+++
alpha-ISOMETHYL IONONE	127-51-5	++
(DL)-LIMONENE	138-86-3	++ (non-ox.); +++ (ox.)
LINALOOL	78-70-6	++ (non-ox.) +++ (ox.)
LINALYL ACETATE	115-95-7	+
MENTHOL	1490-04-6 / 89-78-1 / 2216-51-5	++
6-METHYL COUMARIN [#]	92-48-8	++ (photo-allergy)
METHYL 2-OCTYNOATE	111-12-6	++
METHYL SALICYLATE	119-36-8	+
3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL	67801-20-1	++ (r.t.)
alpha-PINENE and beta-PINENE	80-56-8 and 127-91-3, resp.	++
PROPYLIDENE PHTHALIDE	17369-59-4	+ (r.t.)
SALICYLALDEHYDE	90-02-8	++

[#] 76/768/EEC Annex III, part 1

[#] 76/768/EEC Annex III, part 1

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment: see text
alpha-SANTALOL and beta-SANTALOL	115-71-9 and 77-42-9, resp.	++
SCLAREOL	515-03-7	+
TERPINEOL (mixture of isomers)	8000-41-7	+
alpha-TERPINEOL	10482-56-1 / 98-55-5	
Terpinolene	586-62-9	++
TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	+
TRIMETHYL-BENZENEPROPANOL (Majantol)	103694-68-4	++
VANILLIN	121-33-5	++

Those substances which were categorised as +++ or more, i.e. those with the most reported cases, were also the top ranking substances in large series of patients tested with the 26 labelled fragrance ingredients ((44, 74) and additionally (170)). Geraniol is an exception, as it was all negative in the Danish study (170), but was still among the top ten in the Dutch and German studies (44, 74), with prevalences of 0.5%-0.6% positives. Geraniol has, in addition, caused many cases of contact allergy in other areas of Europe (49).

The use of absolute numbers allows the pooling of studies with different selection criteria. Limonene and linalool were not tested in their oxidized forms in the three studies (44, 74, 170) and would not have been identified, if only these publications had been the basis of assessment.

It should be noted that oxidised fragrance terpenes with defined content of the major haptens formed after autoxidation have not been commercially available for testing in dermatology clinics. In the published clinical studies testing oxidised fragrance terpenes, the patch test preparations have been obtained specifically for the performed multicentre studies. From 2012, patch test preparations of oxidised limonene and oxidised linalool with defined content of the major allergens in the oxidation mixtures, i.e. the hydroperoxides, are commercially available (see also chapter 5).

Table 7-2 lists those substances which gave rise to a few reported cases of contact sensitisation only, or where results have been reported from just one clinical department. Thus, the level of evidence concerning human data must be regarded as *limited*, according to the definitions given in chapter 6.3.

Table 7-2: Fragrance substances with positive human data, which are, however, not sufficient to categorise as “established contact allergen in humans”. More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment	Ref.
AMBRETTOLIDE	7779-50-2	3.4% positive reactions in 178 patients	(171)
CARVACROL	499-75-2	2 of 28 patients	(Meynadier, after (172))
CUMINALDEHYDE	122-03-2	3 of 179 patients positive	(139)
CYCLOHEXYL ACETATE	622-45-7	0.5% positive of 218 selected patients	(173)
CYCLOPENTADECANONE	502-72-7	3 of 178 patients positive	(171)
trans-trans-delta-DAMASCONE	71048-82-3	1 positive HRIPT (2/15 with 1%)	(174)
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	116-26-7	1 positive HRIPT (5 of 53)	(175).
DIMETHYLTETRAHYDRO BENZALDEHYDE	68737-61-1	2.3% positive reactions isomer mixture in 178 patients	(171)
ETHYLENE DODECANEDIOATE	54982-83-1	2 / 218 positive PT reactions	(173)
ETHYL VANILLIN	121-32-4	1 occupational case	(176)
HELIOTROPINE	120-57-0	6 / 1606 consecutive patients positive	(97)
HYDROXYCITRONELLOL	107-74-4	6.0% positive PT reactions in 218 patients	(173)
ISOAMYL SALICYLATE	87-20-7	1 positive in 179 patients, possibly “excited back syndrome” 0 / 95 in another study with <= 1/10 of above test conc.	(139) (70)
ISOLONGIFOLENEKETONE	33407-62-4	1 / 178 patients	(171)
METHOXYCITRONELLAL	3613-30-7	Positive PT data of unknown validity by Nakayama et al. in 22/137 patients.	(177)
METHOXYTRIMETHYLHEPTANOL	41890-92-0	0.9% positive PT	(173)
METHYL p-ANISATE	121-98-2	1 / 182 patients positive	(178)
METHYL CINNAMATE	103-26-4	6 / 142 patients positive	(179)
METHYL DIHYDROJASMONATE	24851-98-7	3 / 1606 patients positive 0 / 100	(97) (70)
METHYLIONANTHEME	55599-63-8	1 case	(180)
5-METHYL-alpha-IONONE	79-69-6	5 / 1606	(97)
METHYL OCTINE CARBONATE	111-80-8	1 case	(181)

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Comment	Ref.
MYRCENE	123-35-3	1 / 1511 positive to oxidized myrcene	(133)
MYRTENOL	515-00-4	2 HRIPTs with 1 pos. each	(182)
NEROL	106-25-2	6.0% positive	(173)
Nerolidol (isomer not specified)	7212-44-4	Few, unconfirmed pos. cases according to RIFM review	(183)
NOPYL ACETATE	128-51-8	2 / 179 positive, possibly "excited back syndrome"	(139)
PHENETHYL ALCOHOL	60-12-8	1 / 179; 0 / 100	(139) (70)
PHENYLACETALDEHYDE	122-78-1	1.1% of 182 positive. 1 case	(178) (184)
PHENYLPROPANOL	122-97-4	2 / 218	(173)
PHYTOL	150-86-7	1 case in human max. test	(185)
RHODINOL	6812-78-8	Several pos. HRIPTs, clinical data of uncertain validity	(186)
trans-ROSE KETONE-5	39872-57-6	2 / 22 pos. HRIPT	(187)

For a number of substances negative patch tests results were obtained, usually in rather small patient samples (max. 313 patients). For some of these substances exposure is substantial, according to data submitted from IFRA. It should be noted that a negative result does not rule out a notable sensitisation prevalence, as the study size has to be larger than, e.g. n=298 to yield a 95% CI which excludes a prevalence of 1% and larger than n=597 to exclude a prevalence of 0.5%.

Table 7-3: Fragrance substances with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Results / Comment	Ref.
6-ACETYL-1,1,2,4,4,7-HEXAMETHYLTETRALINE	21145-77-7	0 / 313 consecutive patients in 2 centres	(70)
AMYL CYCLOPENTANONE	4819-67-4	0 / 178	(171)
BENZYL ACETATE	140-11-4	0 / 100 consecutive patients in 1 centre observed	(70)
2-TERT-BUTYL CYCLOHEXYL ACETATE	88-41-5	0 / 313 consecutive patients in 2 centres	(70)
4-tert.-Butylcyclohexyl acetate	32210-23-4	0 / 107 consecutive patients in 1 centre observed	(70)
6-ETHYLIDENE OCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	93939-86-7	0 / 178	(171)
3a,4,5,6,7,7a-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	54830-99-8	0 / 313 consecutive patients in 2 centres	(70)
HEXYL SALICYLATE	6259-76-3	0 / 218 "top 100" substance and classified as R43	(173)
HIBISCOLIDE	6707-60-4	0 / 178	(171)
alpha-IONONE	127-41-3	0 / 205	(70)
beta-IONONE	79-77-6	0 / 205 "top 100" substance	(70)
ISOBORNYL ACETATE	125-12-2	0 / 107 "top 100" substance	(70)
METHYL ANTHRANILATE	134-20-3	0 / 91 "top 100" substance	(188)
METHYL IONONE (mixture of isomers)	1335-46-2	0 / 100 "top 100" substance	(70)
OXALIDE	1725-01-5	0 / 178	(171)
TERPINEOL ACETATE (Isomer mixture)	8007-35-0	0 / 106 "top 100" substance	(70)
alpha-TERPINYL ACETATE	80-26-2	0 / 179	(139)
TRIMETHYL-PROPYL CYCLOHEXANEPROPANOL	70788-30-6	0 / 178	(171)

For yet another subset of substances, no human data were publicly available. However, exposure to these substances is important as they are used in high volumes (this being the sole criterion for inclusion in this list) and, therefore their hazard with regard to contact sensitisation should be examined.

Table 7-4: Fragrance substances lacking human data and used in high volumes according to industry information.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number
ANISALDEHYDE	123-11-5
BENZYL ACETONE	2550-26-7
p-tert. -Butyldihydrocinnamaldehyde	18127-01-0
CITRONELLYL NITRILE	51566-62-2
CYCLAMEN ALDEHYDE	103-95-7
alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE	10461-98-0
DECANAL	112-31-2
DIHYDROMYRCENOL	18479-58-8
2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE	68039-49-6
3,7-DIMETHYL-1,6-NONADIEN-3-OL	10339-55-6
DIPHENYL ETHER	101-84-8
ETHYL 2-METHYLBUTYRATE	7452-79-1
2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	28219-61-6
ETHYLENE BRASSYLATE	105-95-3
EUCALYPTOL	470-82-6
GERANYL ACETATE	105-87-3
HEXAHYDRO-METHANOINDENYL PROPIONATE	68912-13-0
HEXYL ACETATE	142-92-7
IONONE isomeric mixture	8013-90-9
ISOAMYL ACETATE	123-92-2
ISOBERGAMATE [#]	68683-20-5
Longifolene	475-20-7
METHYLENEDIOXYPHENYL METHYLPROPANAL	1205-17-0
METHYLBENZYL ACETATE	93-92-5
METHYL DECENOL	81782-77-6
METHYL beta-NAPHTHYL ETHER	93-04-9
METHYLUDECANAL	110-41-8
OXACYCLOHEXADECENONE	34902-57-3
PENTADECALACTONE	106-02-5
PHENETHYL ACETATE	103-45-7
PHENOXYETHYL ISOBUTYRATE	103-60-6
PHENYLISOHEXANOL	55066-48-3
Tetrahydrolinalool	78-69-3
TETRAHYDRO-METHYL-METHYLPROPYL)-PYRAN-4-OL	63500-71-0

[#] Annex III, part 1

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number
TRICHLOROMETHYL PHENYL CARBINYL ACETATE	90-17-5
TRICYCLODECENYL PROPIONATE	17511-60-3
TRIMETHYLHEXYL ACETATE	58430-94-7
gamma-UNDECALACTONE	104-67-6
VERDYL ACETATE	2500-83-6/ 5413-60-5

7.2. Tabular summary of evaluated natural extracts/essential oils

Natural raw materials in terms of extracts are used in the fragrance and flavour industry for various reasons. Most importantly, several naturally occurring mixtures have a very complex composition and sensory nature which cannot (fully) be achieved by synthetic the demand for perfumes based on natural materials is considerable (189).

The three main methods used to concentrate plant fragrance substances (190); distillation, mechanical separation ("pressing"), and solvent extraction, yield very different extracts. Essential oils are obtained by water steam, water, ethanol, or water/ethanol distillation. Essence oils are essential oils that separate from the aqueous phase in the distillation receiver during the distillative concentration of fruit, usually citrus, juices. Citrus peel oils, apart from distilled lime oil, are prepared in a special way by pressing the peel to release mostly volatile substances from the pericarp in small oil glands, mostly highly volatile terpene hydrocarbons. However, they also contain small amounts of non-volatile compounds such as dyes, waxes and furocoumarins. The method of solvent extraction is generally applied in the separation of heat-labile materials or if an essential oil can only be obtained in very low yield, e.g. from blossoms. It is also used if the non-volatile components are desired for their fixative properties, e.g. in the preparation of resinoids from exudates. The most important extracts are termed: (i) concretes, an extract of fresh plant material with nonpolar solvents, containing not only volatile, but also a large proportion of non-volatile substances such as waxes; and (ii) absolutes, which are prepared by taking up concretes in ethanol; compounds that precipitate on cooling are removed by filtration, yielding a wax-free residue called absolute. Resinoids, used for their fixative properties, are prepared by extracting plant exudates with alcohols or nonpolar solvents. The products are usually highly viscous and thus sometimes diluted, e.g. with phthalates or benzyl benzoate. Oleoresins are concentrates prepared from spices by solvent extraction (189).

An ISO norm exists regarding the nomenclature of aromatic natural raw materials (ISO/DIS 9235 Aromatic raw materials - vocabulary; International Standardisation Organisation, Geneva, Switzerland). This nomenclature has been considered in Annex I, whereas in the present opinion, nomenclature is according to the CosIng database. Concerning extraction processes for many essential oils, ISO standards exist; for detailed information see Annex I to this opinion.

Regarding clinical data in terms of contact allergy to essential oils and natural extracts, the main focus is on general dermatological patients with complaints related to use of cosmetics etc. However, series of cases with occupational exposure to essential oils with occupational allergic contact dermatitis have also been reported (e.g. masseurs, physiotherapists (191, 192), aromatherapists (193-197), beauticians performing massages (198). For further details, e.g. PT results with various essential oils, see Annex I.

In this section, a tabular overview on the classification of substances considered is presented in three tables listing:

1. Extracts identified as *established contact allergens* in humans(→ *sufficient human evidence present*).

2. Extracts with positive human data, which are, however, not sufficient to categorise as *established contact allergen* in humans (→ *limited human evidence present*).
3. Extracts with negative human data, i.e. patch tests of patients with suspected contact allergy to fragrance ingredients which yielded negative results.

In Table 7-5, essential oils with sufficient human evidence to categorise these as *established contact allergens* in humans are presented.

Table 7-5: Natural extracts classified as established contact allergens in humans (summary of evaluation as detailed in chapter 6.3). More detailed information forming the basis of this evaluation can be found in Annex I of this opinion, including variants of botanical nomenclature.

INCI name (or, if none exists, ^{\$} perfuming name according to CosIng ⁴) in bold; plant part / type of extract (partly indicative) in plain font	CAS number	Comment: see text
CANANGA ODORATA and <i>Ylang-ylang oil</i>	83863-30-3; 8006-81-3	+++
CEDRUS ATLANTICA BARK OIL	92201-55-3; 8000-27-9	++
CINNAMOMUM CASSIA LEAF OIL CINNAMOMUM ZEYLANICUM BARK OIL	8007-80-5 84649-98-9	++ (r.t.)
CITRUS AURANTIUM AMARA FLOWER / PEEL OIL	8016-38-4; 72968-50-4	++
CITRUS BERGAMIA PEEL OIL EXPRESSED ^{\$}	89957-91-5	+ (r.t.)
CITRUS LIMONUM PEEL OIL EXPRESSED [#]	84929-31-7	++
CITRUS SINENSIS (syn.: <i>AURANTIUM DULCIS</i>) PEEL OIL EXPRESSED ^{\$}	97766-30-8; 8028-48-6	++
CYMOPOGON CITRATUS / SCHOENANTHUS OILS	89998-14-1; 8007-02-1; 89998-16-3	++
EUCALYPTUS SPP. LEAF OIL ^{\$}	92502-70-0; 8000-48-4	++
EUGENIA CARYOPHYLLUS LEAF / FLOWER OIL	8000-34-8	+++
EVERNIA FURFURACEA EXTRACT ⁵ (Tree moss)	90028-67-4	+++
EVERNIA PRUNASTRI EXTRACT (Oak moss) [#]	90028-68-5	+++
JASMINUM GRANDIFLORUM / OFFICINALE	84776-64-7; 90045-94-6; 8022-96-6	+++
JUNIPERUS VIRGINIANA	8000-27-9; 85085-41-2	++
LAURUS NOBILIS	8002-41-3; 8007-48-5; 84603-73-6	++
LAVANDULA HYBRIDA	91722-69-9	+ (r.t.)
LAVANDULA OFFICINALIS ^{\$}	84776-65-8	++
MENTHA PIPERITA	8006-90-4; 84082-70-2	++
MENTHA SPICATA	84696-51-5	++
MYROXYLON PEREIRAE (Balsam of Peru) [#]	8007-00-9	++++

⁴ <http://ec.europa.eu/consumers/cosmetics/cosing/>

[#] 76/768/EEC Annex III, part 1

[#] 76/768/EEC Annex III, part 1

INCI name (or, if none exists, ^{\$} perfuming name according to CosIng ⁴) in bold; plant part / type of extract (partly indicative) in plain font	CAS number	Comment: see text
NARCISSUS SPP.	<i>diverse</i>	++
PELARGONIUM GRAVEOLENS	90082-51-2; 8000-46-2	++
PINUS MUGO/ PUMILA [#]	90082-72-7 / 97676-05-6	++
POGOSTEMON CABLIN	8014-09-3; 84238-39-1	++
ROSE FLOWER OIL (ROSA SPP.)	<i>Diverse</i>	++
SANTALUM ALBUM	84787-70-2; 8006-87-9	+++
TURPENTINE (oil) [#]	8006-64-2; 9005-90-7; 8052-14-0	++++
VERBENA absolute [#]	8024-12-2	++

Notes: r.t. Rarely tested.

Table 7-6 lists a number of essential oils, mostly tested in just one clinical department, and thus, or for other reasons, not satisfying the criteria for being categorised as *established contact allergen* in humans (i.e. *limited human evidence present*).

Table 7-6: Natural extracts with positive human data, which are, however, not sufficient to categorise as “established contact allergen in humans”. More detailed information forming the basis of this evaluation can be found in Annex I of this opinion.

INCI name (or, if none exists, perfuming name according to CosIng) in bold; plant part / type of extract (partly indicative) in plain font	CAS number	Comment	Ref.
ACORUS CALAMUS ROOT OIL	84775-39-3	n=7 pos. reactions to “calamus”	(199)
CEDRUS DEODARA WOOD OIL	91771-47-0	Rudzki 1976/1986 found 3 / 3 positive reactions	(199, 200).
CITRUS AURANTIUM AMARA LEAF OIL	72968-50-4	Several cases in 2 series from 1 centre	(199, 200)
CITRUS TANGERINA ...	223748-44-5	1 case	(201)
CYMBOPOGON NARDUS / WINTERIANUS HERB OIL	89998-15-2; 91771-61-8	Several cases in 2 series from 1 centre	(199, 200)
ILLICIUM VERUM FRUIT OIL	84650-59-9	Cases of active sensitisation; 34% consecutive patients pos. to 1%	(202)

INCI name (or, if none exists, perfuming name according to CosIng) in bold; plant part / type of extract (partly indicative) in plain font	CAS number	Comment	Ref.
LAVANDULA SPICA	97722-12-8	Several cases in 2 series from 1 centre	(199, 200)
LITSEA CUBEBA	90063-59-5	Several cases in 2 series from 1 centre	(199, 200)
PELARGONIUM ROSEUM	90082-55-6	2.1% pos. of 1483 patients	(203)
ROSMARINUS OFFICINALIS	84604-14-8	3 cases in 2 series from 1 centre	(199, 200)
SALVIA spp.	Diverse	Several cases in 2 series from 1 centre	(199, 200)
TAGETES PATULA	91722-29-1	1 case (aromatherapist)	(193)
THYMUS spp.	84929-51-1	4 / 84 pos	(199)
VETIVERIA ZIZANOIDES	8016-96-4; 84238-29-9	1 / 200 and 9 / 86 pos.	(199, 200)

The final table is an indicative list of natural extracts which lack published human data, but which are of interest: (i) as high-volume exposure; (ii) due to published positive animal experiments; or (iii) because they contain well-known (established) contact allergens.

Table 7-7: Indicative list illustrating natural extracts containing established human allergens or having R43-label or positive LLNA, lacking published human data.

INCI name (or, if none exists, perfuming name according to CosIng) in bold; plant part / type of extract (partly indicative) in plain font	CAS number	Comment
CITRUS PARADISI PEEL OIL	8016-20-4	high volume substance, classified as R43
CYMBOPOGON MARTINI HERB EXTRACT	84649-81-0	Pos. LLNA study by RIFM: EC3 value 9.6% (204).
MENTHA ARVENSIS	68917-18-0	high volume, classified as R43
OCIMUM BASILICUM	84775-71-3	Pos. LLNA study by RIFM: EC3 value < 2.5% (204).
PIMENTA RACEMOSA	85085-61-6	Contains, among other substances, the established contact allergen eugenol (42-56%)
SANTALUM SPICATA	8024-35-9	Contains, among other substances, the established contact allergens santalols (75%) and farnesol (10%)

7.3. Conclusions

- According to the criteria described in chapter 6.3 a total of 54 individual chemicals and 28 natural extracts (essential oils) can be categorised as *established contact allergens* in humans, including all currently regulated substances.
- Of the 54 individual chemicals which are established contact allergens in humans, 12 are considered to be of special concern due to the high number of reported cases, (> 100, i.e. category +++ or ++++ in Table 7-1). These are further considered in chapter 5 (limonene and linalool) and the remainder in chapter 11. In particular one ingredient stands out, hydroxyisohexyl 3-cyclohexene carboxaldehyde, having been the cause of more than 1,500 reported cases since the 1999 opinion (see also chapter 4.3.1, chapter 11.3 and Annex I).
- For an additional 33 individual chemicals (Table 7-2) and 14 natural extracts (Table 7-6), positive patch test results have been reported. However, they do not qualify for the above category, i.e. only *limited human evidence* is present.
- For a number of fragrance substances (n=18, Table 7-3) patch testing did not yield positive results. However, numbers of patients tested are generally too small to rule out the existence of clinical contact sensitisation with sufficient confidence. No clinical evidence has been identified for 39 individual chemicals that have been reported to be frequently used (Table 7-4).
- For the substances (and, if possible, also for the main constituents of the natural mixtures) with limited or no human evidence, additional animal data and/or SAR considerations are taken into account. Aggregated data for these substances are presented in chapter 13.

8. Animal data

8.1. Predictive tests and sensitising potency categories

The animal test methods used in harmonised classification of substances, according to their potential to cause skin sensitisation, are the guinea pig maximisation test (GPMT), the Buehler test⁶ and the local lymph node assay (LLNA)⁷. These methods are used in hazard identification and risk assessment for regulatory purposes under REACH⁸. For registration in REACH, the LLNA is the preferred method for measuring skin sensitisation potential in animals, and justification for the use of other methods needs to be provided. According to the directives on classification and labelling⁹, substances and preparations meeting positive criteria in these tests shall be classified as sensitising and assigned the symbol "Xi" and the risk phrase "R43: May cause sensitisation by skin contact"; or, according to the recent regulation on classification, labelling and packaging (CLP¹⁰) "H317: May cause an allergic skin reaction".

As yet, there is no officially validated *in vitro* test method for skin sensitisation. Therefore, for cosmetic ingredients the LLNA, the GPMT and the Buehler test have also been used in risk assessment for regulatory purposes.

Positive results from the OECD guideline animal tests mentioned above which are sufficient to classify a substance as a skin sensitiser (R43) are:

- GPMT; at least 30% of the animals have a positive response.
- Buehler test; at least 15% of the animals have a positive response.
- LLNA; at least a 3-fold increase in lymph node cell proliferative activity is induced, compared to vehicle-treated controls (stimulation index $SI \geq 3$). For positive LLNAs, an EC3 value is calculated which gives the estimated concentration of a chemical necessary to give a 3-fold increase in proliferative activity compared to vehicle-treated controls.

Further categorisation of substances classified with R43 into three groups according to allergen potency (extreme, strong and moderate) has been proposed by a European Commission expert group on skin sensitisation (161, 205), and proposed also in the ECHA guidance document on application of the CLP criteria (162). Such categorisation is based on EC3 values in the LLNA, on intradermal induction concentration in the GPMT, and topical induction concentration in the Buehler test. The potency categories and their default concentration values based on EC3 values in the LLNA as defined in (161): extreme sensitiser (EC3 value ≤ 0.2); strong sensitiser (EC3 $> 0.2 - \leq 2$); and moderate sensitiser (EC3 value > 2). When LLNA EC3 values are available from more than one study, the lowest value should normally be used. Where multiple animal data sets lead to different categorisation of the same substance, the higher potency category should apply (161, 205).

The potency categorisation of substances based on the LLNA is applied by the SCCP in risk assessment of cosmetic ingredients, particularly hair dye substances (206).

⁶ OECD Guideline for testing of chemicals. Guideline 406: Skin Sensitisation. OECD, Adopted 12 May 1981, updated 17th July 1992.

⁷ OECD Guideline for testing of chemicals. Guideline 429: Skin Sensitisation: Local Lymph Node Assay. OECD, Adopted 22 July 2010.

⁸ Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

⁹ Directives 67/548/EEC and 1999/45/EC.

¹⁰ Regulation No. 1272/2008.

8.1.1. LLNA data

The SCCS requested the International Fragrance Association (IFRA) to submit data on animal tests performed with fragrance substances, to be presented in a structured format. In response, IFRA submitted first a poster (163) and later a report consisting of LLNA protocol summaries on the 59 fragrance substances in the poster (164). No guinea pig studies were submitted. The SCCS has reviewed and analysed the report and the publications quoted in the report.

Table 8-1 displays the EC3 values for fragrance substances in the report submitted by industry (164). EC3 values for some additional fragrance substances in two published reviews (165, 166) have also been included in Table 8-1. Table 8-2 presents LLNA results for oxidised substances. Full data are given in Annex II. Table 8-3 summarises the distribution of fragrance substances, by potency category, according to EC3 values.

Additional EC3 values may be available in the scientific literature. Many more animal experiments may have been performed, but have not been published.

Table 8-1: Summary of local lymph node assay (LLNA) data on 66 fragrance substances, based on a report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009 (164)) and in published reviews by Gerberick et al. 2005 (165) and Kern et al. 2010 (166), respectively. EC3 values (% and M) are given. The order of substances is by decreasing sensitisation potency as assessed by LLNA EC3 values (lowest EC3 value indicating highest potency).

Substance	CAS no.	EC3 value		Reference
		%	M	
Hexyl salicylate	6259-76-3	0.18	0.008	(164, 166)
Cinnamal	104-55-2	0.2	0.015	(164)
Methyl 2-octynoate	111-12-6	<0.5	<0.032	(164, 166)
Isoeugenol	97-54-1	0.54	0.033	(164)
Citral	5392-40-5	1.2	0.079	(164)
2-Hexylidene cyclopentanone	17373-89-6	2.4	0.14	(164)
Methyl octine carbonate	111-80-8	2.5	0.15	(164)
Peru balsam absolute	8007-00-9	2.5	n/a	(164)
trans-2-Hexenal	6728-26-3	2.6	0.26	(164)
Benzyl Salicylate	118-58-1	2.9	0.23	(164, 166)
Butylphenyl methylpropional (BMHCA)	80-54-6	2.9	0.14	(164)
Phenylacetaldehyde	122-78-1	3	0.25	(164, 165)
Allyl phenoxyacetate	7493-74-5	3.1	0.16	(164)
Benzylideneacetone	122-57-6	3.7	0.25	(165)
3-Propylidenephthalide	17369-59-4	3.7	0.21	(164, 165)
<i>Evernia prunastri</i> extract oak moss	90028-68-5	3.9	n/a	(164)
Balsam oil, Peru (<i>Myroxylon pereirae</i> Klotzsch)	8007-00-9	4	n/a	(164)
Farnesol	4602-84-0	4.1	0.18	(164)
p-t-Butyl-dihydrocinnamaldehyde	18127-01-0	4.3	0.23	(164)
α-Methyl cinnamic aldehyde	101-39-3	4.5	0.31	(164, 165)

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Substance	CAS no.	EC3 value		Reference
		%	M	
Eugenol	97-53-0	5.3	0.32	(164)
Hexyl cinnamal	101-86-0	5.3	0.25	(164)
Dihydrocoumarin	119-84-6	5.6	0.38	(165)
Geraniol	106-24-1	5.6	0.36	(164)
Carvone	6485-40-1	5.7	0.38	(164)
Diethyl maleate	141-05-9	5.8	0.34	(165)
2-Methoxy-4-methylphenol	93-51-6	5.8	0.42	(164, 165)
Anise alcohol	105-13-5	5.9	0.43	(164, 166)
Jasmine absolute (<i>Grandiflorum</i>)	8022-96-6	5.9	N/a	(164)
Dibenzyl ether	103-50-4	6.3	0.32	(164)
<i>Cananga odorata</i> leaf/flower oil ylang ylang "extra"	8006-81-3	6.8	N/a	(164)
Isocyclocitral	1335-66-6	7.3	0.48	(164)
2,3-Dihydro-2,2,6-trimethylbenzaldehyde	116-26-7	7.5	0.50	(165)
Amyl cinnamal	122-40-7	7.6	0.38	(164)
Perillaldehyde p-Mentha-1,8-dien-7-al	2111-75-3	8.1	0.54	(164, 165)
p-Isobutyl- α -methyl hydrocinnamaldehyde	6658-48-6	9.5	0.46	(164)
d-Limonene*	5989-27-5	<10	<0.73	(164)
Methylundecanal	110-41-8	10	0.54	(165)
Acetylcedrene	32388-55-9	13.9	0.57	(166)
Methylenedioxyphenyl methylpropanal	1205-17-0	16.4	0.85	(164, 166)
Benzyl benzoate	120-51-4	17	0.80	(165)
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	31906-04-4	17.1	0.81	(164, 165)
Benzyl cinnamate	103-41-3	18.4	0.77	(164, 166)
Hydroxycitronellal	107-75-5	19.3	1.12	(164)
Cinnamyl alcohol	104-54-1	21	1.57	(165)
α -iso-Methylionone	127-51-5	21.8	1.06	(164, 166)
Cyklamen aldehyde	103-95-7	22	1.64	(165)
4-Methoxy- α -methyl benzenpropanal	5462-06-6	23.6	1.32	(164)
Amyl cinnamyl alcohol	101-85-9	~25	~1.22	(164, 166)
Tetramethyl acetyloctahydronaphthalenes (OTNE)	54464-57-2	25.1	1.07	(164)
Ethyl acrylate	140-88-5	28	2.8	(165)
Linalool*	78-70-6	30	1.94	(165)
Trimethylbenzenepropanol Majantol	103694-68-4	30	~1.68	(164)
Jasminum Sambac Flower CERA/Extract/Water	91770-14-8	35.4	N/a	(164)

Substance	CAS no.	EC3 value		Reference
		%	M	
Citronellol	106-22-9	43.5	2.78	(164, 166)
No EC3 value was established; higher concentrations should also have been tested				
6-Methyl-3,5-heptadien-2-one	1604-28-0	>5	>0.40	(164)
<i>Camellia sinensis</i> leaf tea leaf absolute	84650-60-2	>5	N/a	(164)
Cinnamyl nitrile	1885-38-7	>10	>0.77	(164)
Menthadiene-7-methyl formate	68683-20-5	>10	>0.51	(164)
<i>Evernia furfuracea</i> extract tree moss absolute	90028-67-4	>20	N/a	(164)
Isocyclogeraniol	68527-77-5	>25	>1.62	(164)
1-Octen-3-yl acetate	2442-10-6	>30	>1.76	(164)
Benzyl alcohol	100-51-6	>50	>4.62	(164)
Coumarin	91-64-5	>50	>3.42	(164)
Vanillin	121-33-5	>50	>3.3	(164)
No EC3 value calculated				
Benzaldehyde	100-52-7	-		(165)

Notes: * Material with low levels of oxidation according to (164)

n/a: Not applicable (mixture of compounds).

M: EC3 based on molar concentration

8.1.2. LLNA data on oxidised fragrance substances

For fragrance substances that can autoxidise upon air exposure, it is also important to investigate the sensitisation potency after air exposure. The oxidised compounds are clinically relevant as they represent what the consumers could come in contact with from perfumes and fragranced products. In Table 8-2 the LLNA data for some of the most commonly used fragrance substances, pure and after autoxidation, are presented. The EC3 values obtained for the pure substances are 5-10 times higher compared to those obtained for the same substances after air exposure. The experimental air exposure simulated air exposure that can take place during normal handling and storage. In the production process, some perfumes are "matured" aerobically, stirring included. During this process, some fragrance substances may be oxidised. It should be noted that, although only a few substances capable of oxidation have so far been investigated, structural alerts indicating possible autoxidation are common among the fragrance substances listed in this document (see chapter 9). It is important to further investigate this issue for increased understanding of the associated risk.

Table 8-2: Local lymph node assay (LLNA) data on four fragrance substances and one essential oil before and after air exposure, comparing the sensitisation potency of the pure (not oxidised) substance with the potency of the oxidised.

Substance	CAS no.	Doses % (w/v) vehicle: A:OO 4:1*	EC3 value (% w/v)	Reference
D-Limonene (ox. 10 w)	5989-27-5	1, 5, 25	3.0	(207)
D-Limonene (pure)	5989-27-5	25, 50, 100	30	

Substance	CAS no.	Doses % (w/v) vehicle: A:OO 4:1*	EC3 value (% w/v)	Reference
Linalool (ox. 10 w)	78-70-6	5, 10, 25	9.4	(127)
Linalool (ox. 45 w)	78-70-6	2.5, 10, 25	4.8	
Linalool (pure)	78-70-6	25, 50, 100	46.2	
Linalyl acetate (ox. 10 w)	115-95-7	0.5, 10, 40	3.6	(128)
Linalyl acetate (pure)	115-95-7	10, 30, 100	25	
Geraniol (ox. 10 w)	106-24-1	1, 3, 6, 10, 20	4.4	(119)
Geraniol (ox. 45 w)	106-24-1	0.5, 1, 3, 6, 10	5.8	
Geraniol (pure)	106-24-1	5, 10, 15, 20, 30	22.4	
Lavender oil (ox. 10 w)		1, 5, 10, 20, 50	11	(140)
Lavender oil (ox. 45 w)		1, 5, 10, 20, 50	4.4	
Lavender oil (not ox.)		5, 25, 100	36	

Notes: Pure: Purified before testing as most commercially available fragrance substances are not pure.

Not ox.: Not purified but used as it was delivered as this is a complex mixture and not a specific substance.

Ox. x w: Oxidised by air exposure during x weeks.

* Acetone:olive oil.

8.2. Methodological considerations

EC3 mean values

In the submitted poster (163) and the report by IFRA (164), the LLNA weighted mean EC3 values ($\mu\text{g}/\text{cm}^2$) are presented. The SCCS considers it is misleading to present EC3 values as mean values from tests performed with different vehicles. It is generally agreed that the lowest EC3 value should be used if there is more than one study fulfilling the OECD guideline requirements (161, 205), and these have been introduced into Table 8-1. The EC3 values in the reviews by Gerberick et al. and Kern et al. (165, 166) were based on single representative experiments with a vehicle described in the OECD guideline 429 (see above), and preferably with acetone:olive oil. EC3 mean values, as in the submission by IFRA, were not presented in these two reviews.

Vehicle

The most frequently used *vehicle* in the submission by IFRA (164) was ethanol:diethyl phthalate (1:3), followed by acetone:olive oil (4:1). In some experiments, antioxidants were mixed with ethanol:diethyl phthalate. The vehicle was not reported in some of the references, and no rationale for using vehicles other than those recommended was given in the report (164). According to the OECD guideline 429 (see above), the recommended vehicles are acetone:olive oil (4:1), N,N-dimethylformamide, methyl ethyl ketone, propylene glycol, and dimethyl sulphoxide, but others may be used if sufficient scientific rationale is provided. It is well known that a difference in the EC3 value can be obtained for the same substance depending on which vehicle is used in the LLNA. Thus as an *additional control*, supplementary to the guideline based LLNA control, a clinically relevant solvent or the commercial formulation in which the test substance is marketed may be used.

Number of doses and animals

According to the OECD guideline 429 (see above), a minimum of three concentrations should be tested. The number of consecutive doses used in the reported data, was generally five, sometimes three and in few experiments two. The SCCS considers that too few concentrations were tested in four studies in which only two concentrations were used. Lower concentrations than those tested should have been used in experiments with five fragrance substances, in which the EC3 value could not be determined. Higher concentrations than those tested should also have been used in experiments with 12 substances, in which the EC3 value could not be determined.

The *number of animals* per dose group was generally four plus a non-exposed control group, sometimes five, and in few experiments six; the minimum according to the OECD guideline being four.

Units for concentrations

In the submission by IFRA (164) the EC3 values are given in weight per area unit ($\mu\text{g}/\text{cm}^2$). The SCCS considers that the EC3 values (%) are the values of primary interest in communicating risk assessment, as EU legislation, OECD guideline 429 and scientific literature refer to EC3 values (%). However, the SCCS recommends that molar (M) EC3 values should be considered, as they give the concentration based on the molecular weight of substances. They have thus been calculated and introduced into Table 8-1.

EC3 values (%) overestimate the intrinsic molecular sensitisation potency for low molecular weight compounds while compounds with a high molecular weight are underestimated. Regarding the differences in molecular weight between the studied fragrance substances, a variation is seen if the ranking list of the sensitisation potency is based on EC3 (%) or EC3 (M) since some substances have a molecular weight twice as high as others.

From comparisons in Table 8-1, we notice that, e.g. hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has an EC3 value of 17.1 %, or 0.81 M when the calculation includes its molecular weight, while for trans-2-hexenal the corresponding values are 2.6% and 0.26 M. The example shows that comparing the sensitisation potency between these two substances using the EC3 values in % exaggerates the sensitisation potency of trans-2-hexenal compared to that of HICC. When using the EC3 values in molar concentrations the difference is not so pronounced.

8.3. Summary of animal data by LLNA

The distribution of sensitising potency of fragrance substances compared to other substances, (e.g. biocides, dyes, plastic materials) taken from three references (164-166) as assessed by EC3 values in the LLNA, is shown in Figure 8-1 and Table 8-3.

For 10 substances, no EC3-value could be established. These should have been tested at higher concentrations – some of these would most probably have generated an EC3 value. However, we reported here “No EC3 value established”. 5 substances should have been tested also at lower concentration and in these cases the EC3 value could have been lowered, meaning a more severe potency category could have been achieved. In all, approx 150 experiments were reported in (164), listed in Annex II.

The median EC3 value of evaluable fragrance substances (5.9%) is similar to other substances tested (5.5%). However, very few fragrance substances have low EC3 values (≤ 2).

Substances with an EC3 value ≤ 2 may be categorised as strong or extreme sensitisers. Such potent sensitisers are comparatively rare among fragrance substances assessed in the LLNA. Nevertheless, fragrances are important allergens in humans, which points to repeated skin exposure to less potent sensitisers as a factor strongly determining sensitisation risk.

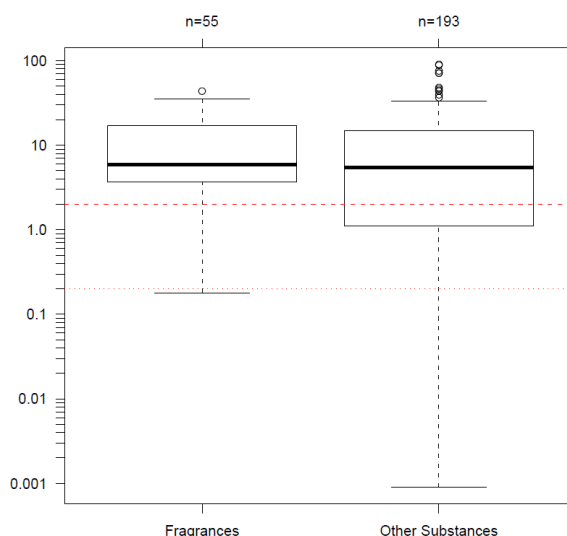


Figure 8-1: The distribution of fragrance chemicals and a variety of other chemicals (e.g. biocides, dyes, plastic materials), taken from the three references (164-166), are depicted as boxplots on a logarithmic scale. The bottom of the box denotes the 1st quartile (25% percentile), the thick line in the box the median, and the top of the box the 3rd quartile (75% percentile). Outliers, i.e. below the 25% and above the 75% percentiles, are shown as whiskers. Beyond the 1.5-fold interquartile range, single values are shown as circles instead of whiskers. The difference in distribution is not significant (Wilcoxon test: $p=0.061$).

Note: EC3 values for the five oxidised fragrances additionally examined (Table 8-2) range from 3.0 to 4.8 (median 4.4) and are lower by a factor of around 7 than EC3 values of the respective non-oxidised material.

Table 8-3: Summary of EC3 values for fragrance substances in Table 8-1 and for other substances, all taken from the three references (164-166). The EC3 value intervals for potency categorisation (161, 205) were used for comparison of fragrances substances vs other substances.

EC3 value interval	Fragrance substances		Other substances	
	n	%	n	%
≤ 0.2	2	3%	28	11%
$> 0.2 - \leq 2$	3	4%	38	15%
> 2	50	71%	127	49%
No EC3 value established *	10	14%	0	0%
No EC3 value calculated (NC)	5	7%	69	26%
All substances	70		262	

Note: * Substances should have been tested also at higher concentrations.

8.4. Conclusions

- In the event that human data are lacking, the LLNA provides important information on skin sensitising potential and potency.
- Animal data on fragrance substances submitted by IFRA (164) and assessed in this opinion were generated exclusively by LLNA. Other guideline methods are, however, also available.
- The vast majority of the submitted (164) and additional (165, 166) fragrance substances tested by the LLNA are skin sensitisers.
- Several studies in the IFRA report (164) were of insufficient quality, not following the OECD guideline.

- Fragrance substances that can be predicted to autoxidise upon air exposure should also be tested after air exposure, as oxidation may significantly increase their sensitising potency.
- It can be concluded that the skin sensitising potency, as assessed by the LLNA, is only one of several factors that are of importance for sensitisation to fragrance substances. This is illustrated by the fact that only a small fraction of sensitising fragrance substances can be categorised as an extreme allergen based on LLNA test results. Therefore, doses from repeated deposition onto skin must be considered a driving force of sensitisation risk.

9. Structure activity relationships (SAR): grouping of substances based on expert judgement

Whether or not a particular chemical will be a sensitiser, and how potent it will be if it is a sensitiser, depends on its ability, either directly or after activation, to react with appropriate proteins in the skin. This fundamental concept was initially demonstrated by Landsteiner and Jacobs in 1936 (208) and subsequently validated by numerous studies with various types of chemicals (some key references: (209-213)). The ability to predict sensitisation potency, or lack of it, depends on being able to predict reactivity to skin proteins. This is the basis of SAR analysis for skin sensitisation. The prediction can often be made based on the chemical structure, recognising structural features (referred to as **structural alerts**) that are associated with reactivity.

The relationships between molecular structure and reactivity that form the basis for structural alerts are based on well established principles of mechanistic organic chemistry (214). Examples of structural alerts are aliphatic aldehydes (alerting to the possibility of sensitisation via a Schiff base reaction with protein amino groups), and α,β -unsaturated carbonyl groups, C=C-CO- (alerting to the possibility of sensitisation via Michael addition of protein thiol groups). Major mechanistic reactivity domains have been discussed in detail by Aptula and Roberts (215). Prediction of the sensitisation potential of compounds that can act via abiotic or metabolic activation (pre- or prohaptens) is more complex compared to that of compounds that act as direct haptens without any activation. The autoxidation patterns can differ due to differences in the stability of the intermediates formed, e.g. it has been shown that autoxidation of the structural isomers linalool and geraniol results in different major haptens/allergens. Moreover, the complexity of the prediction increases further for those compounds that can act both as pre- and prohaptens. In such cases, the impact on the sensitisation potency depends on the degree of abiotic activation (e.g. autoxidation) in relation to the metabolic activation.

These structural alerts can be applied by computerized expert systems, i.e. *in silico* or by estimations made by organic chemists (*in cerebro*) using their experience. When an organic chemist looks at a chemical structure, they recognise parts of the structure that they can associate with reactivity, the type of reactivity (i.e. assign the reaction mechanistic domain), and other features of the molecular structure that will affect the reactivity positively or negatively. Human experts should be aware of the complexities, and how structural modification can alter the reactivity associated with structural alerts, etc. Importantly, they can also recognise where there are unfamiliar structural features whose effects they cannot confidently predict. In such cases they can call for experimental chemistry work (*in chemico*) to be done to ascertain the presence or nature of, and degree of reactivity. *In chemico* methods include organic chemistry experimentation to identify chemical reaction products from oxidation and/or reaction with model nucleophiles, identification of mechanisms of reaction. In so called *in chemico* reactivity methods, the ability of a specific chemical to react with selected peptides is determined so as to predict the sensitisation potential of the chemical under investigation (216, 217). To make *in chemico* reactivity methods able to predict the activity of prohaptens, the addition of horseradish peroxidase and hydrogen peroxide oxidation system has been tested to model the enzymatic oxidation in the skin (218, 219).

Although computerized expert systems are derived from input by human experts, they are less well able to capture the subtleties of structure reactivity relationships, and they sometimes fail to detect aspects of chemistry that are obvious to organic chemists. Human experts should be aware of the complexities, as well as how structural modification can alter the reactivity associated with structural alerts, etc.

The SAR evaluation made in the section below is based on *in cerebro* alerts applied by organic chemists.

Depending on the type of reactivity (the **reaction mechanistic domain**), it is sometimes possible to make a quantitative prediction of potency in the LLNA, which can be used to predict potency in humans relative to related known human sensitisers. These predictions use quantitative mechanistic models (**QMMs**) based on reactivity expressed quantitatively

by model parameters, and sometimes in combination with hydrophobicity. For example, potency of aliphatic aldehydes and ketones (the Schiff base domain) in the LLNA is modelled by a combination of reactivity and hydrophobicity (220), whereas the LLNA potency of DNCB analogues (the S_NAr domain) is well modelled by reactivity alone (221).

QMMs aiming not only to predict the potential to be a sensitizer but also to predict the potency, promise to be a useful tool in non-animal based risk assessment for skin sensitisation. However, in the field of fragrance substances there are major gaps in our present ability to apply QSAR/QMM. This is largely because many of the fragrance substances of interest have the potential to act via abiotic or metabolic activation (pre- and/or prohaptens, i.e. they themselves are only weak or non-sensitizers, but have the potential to be activated to form more potent sensitizers. Resulting sensitization potency will depend on the extent of activation and the nature of the resulting products. It is possible to apply SAR analysis to identify these plausible possibilities, but QSAR modelling for these cases is not yet developed. However, much progress has been made in identifying structural alerts for the various activation mechanisms that have been recognised. This is reviewed by Karlberg et al. (122).

Chemicals with no structural alerts for direct reactivity, or for known activation mechanisms, and no unfamiliar structural features that might be associated with as yet unidentified activation mechanisms, can be predicted to be non-sensitizing. Chemicals that do have alerts for reactivity (direct or via activation) are not necessarily sensitizers – they may be insufficiently reactive and/or insufficiently hydrophobic.

Substances meeting the inclusion criteria (see chapter 6), for which, however, no categorisation as established contact allergen in humans or established contact allergen in animals was possible, have been assessed for structural alerts. The results are presented in four tables based on the prediction made for the actual substance. The following SAR assessments have been used:

- Predicted sensitizer; structural alerts:
Compounds containing structural alerts comprising direct reactive compounds and for compounds that after specific abiotic or metabolic activation (prohaptens and prehaptens) can be predicted to be sensitizers by structural comparison to known allergens.
- Possible sensitizer; structural alerts:
Compounds containing structural alerts that by comparison to known allergens with similar structures were expected to be less reactive and hence less likely to be sensitizing. Also compounds with structural alerts indicating a possible abiotic or metabolic activation (possible prehaptens or prohaptens) but with no structural data available for comparison, were included in this group. Consequently, a possible sensitizer may turn out to be a non sensitizer when tested in vivo.
- Predicted non-sensitizer (NS); no obvious structural alerts
- Not predictable due to insufficient/conflicting data

Table 9-1: Predicted sensitizers.

Substance (INCI) name	CAS number	Structural alerts
p-tert.-Butyldihydrocinnamaldehyde [§]	18127-01-0	Schiff base
Citronellal	106-23-0	Schiff base and possible prehapten
Citronellyl nitrile	51566-62-2	Possible prehapten
Decanal	112-31-2	Schiff base
3,7-Dimethyl-1,6-nonadien-3-ol	10339-55-6	Prehapten
Geranyl acetate	105-87-3	Prehapten and prohapten

Isoamyl salicylate	87-20-7	Acyltransfer agent
Methyl cinnamate	103-26-4	Michael acceptor
Methylundecanal	110-41-8	Schiff base
Myrcene	123-35-3	Prehaptent
Nerol	106-25-2	Prehaptent and prohaptent
Nerolidol (isomer not specified)	7212-44-4	Possible prehaptent
Oxacyclohexadecanone	34902-57-3	Michael acceptor
Phenethyl salicylate	87-22-9	Acyltransfer agent
trans-Rose ketone-5	39872-57-6	Michael acceptor and possible prehaptent

Note: § Classified as R43.

Table 9-2: Possible sensitisers.

Substance (INCI) name	CAS number	Structural alerts
Ambrettolide	7779-50-2	Possible prehaptent
Amylcyclopentanone	4819-67-4	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
Benzyl acetate	140-11-4	Prohaptent via hydrolysis leading to benzyl alcohol
Carvacrol	499-75-2	Possible prehaptent
Cuminaldehyde	122-03-2	Schiff base and possible prehaptent
alpha-Cyclohexylidene benzeneacetone	10461-98-0	Possible Michael acceptor
Cyclopentadecanone	502-72-7	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
trans-beta-Damascone	23726-91-2	Possible Michael acceptor
trans-trans-delta-Damascone	71048-82-3	Possible Michael acceptor and possible prehaptent
gamma-Damascone	35087-49-1	Possible Michael acceptor and possible prehaptent
Dihydromyrcenol	18479-58-8	Possible prehaptent
2,3-Dihydro-2,2,6-trimethylbenzaldehyde	116-26-7	Possible Michael acceptor and possible prehaptent and possible prohaptent
2,4-Dimethyl-3-cyclohexen-1-carboxaldehyde §	68039-49-6	Schiff base and possible prehaptent
Dimethyltetrahydro benzaldehyde	68737-61-1	Schiff base and possible prehaptent
6-Ethylideneoctahydro-5,8-methano-2H-benzo-1-pyran	93939-86-7	Possible prehaptent
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol	19-61-6	Possible prehaptent
Ethyl vanillin	121-32-4	Complex
Heliotropine	120-57-0	Possible prohaptent
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-inden-5(or 6)-yl	54830-99-8	Possible prehaptent

Substance (INCI) name	CAS number	Structural alerts
acetate		
Hexahydro-methanoindenyl propionate	68912-13-0	Possible prehapten
Ionone isomeric mixture	8013-90-9	Possible Michael acceptor and possible prehapten
alpha-Ionone	127-41-3	Possible Michael acceptor and possible prehapten
beta-Ionone	79-77-6	Possible Michael acceptor
Isobergamate	68683-20-5	Possible prehapten
Isolongifoleneketone	33407-62-4	Schiff base; the combination of reactivity and hydrophobicity may be enough to confer sensitisation
Longifolene [§]	475-20-7	Possible prehapten
Methoxycitronellal	3613-30-7	Schiff base
Methyl decenol	81782-77-6	Possible prehapten
Methyl ionone (mixture of isomers)	1335-46-2	Possible Michael acceptor and possible prehapten
Methylionantheme	55599-63-8	Possible Michael acceptor and possible prehapten
5-Methyl-alpha-ionone	79-69-6	Possible Michael acceptor and possible prehapten
Myrtenol	515-00-4	Possible prehapten
Nopyl acetate	128-51-8	Possible prehapten
Phytol	150-86-7	Possible prehapten and/or prohaptent
Rhodinol	6812-78-8	Possible prehapten
Terpineol acetate (isomer mixture)	8007-35-0	Possible prehapten
alpha-Terpinyl acetate	80-26-2	Possible prehapten
Tricyclodecenyl propionate	17511-60-3	Possible prehapten
Verdyl acetate	2500-83-6/ 5413-60-5	Possible prehapten

Note: [§] Classified as R43.

Table 9-3: Predicted non-sensitisers with no obvious structural alerts.

Substance (INCI) name	CAS number	Structural alerts
6-Acetyl-1,1,2,4,4,7-hexamethyltetraline	21145-77-7	
Benzyl acetone	2550-26-7	Schiff base; the combination of reactivity and hydrophobicity may not be enough to confer sensitisation
2-tert.-Butylcyclohexyl acetate	88-41-5	
4-tert.-Butylcyclohexyl acetate	32210-23-4	
Cyclohexyl acetate	622-45-7	
Diphenyl ether	101-84-8	

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Substance (INCI) name	CAS number	Structural alerts
Ethyl 2-methylbutyrate	7452-79-1	
Ethylene dodecanioate	54982-83-1	
Ethylene brassylate	105-95-3	
Eucalyptol	470-82-6	
Hexyl acetate	142-92-7	
Hibiscolide	6707-60-4	
Hydroxycitronellol	107-74-4	However, dehydration followed by autoxidation could give sensitising impurities
Isoamyl acetate	123-92-2	
Isobornyl acetate	125-12-2	
Methoxytrimethylheptanol	41890-92-0	
Methyl p-anisate	121-98-2	
Methyl anthranilate	134-20-3	
Methylbenzyl acetate	93-92-5	
Methyl dihydrojasmonate	24851-98-7	Schiff base; the combination of reactivity and hydrophobicity may not be enough to confer sensitisation
Oxalide	1725-01-5	
Pentadecalactone	106-02-5	
Phenethyl acetate	103-45-7	
Phenethyl alcohol	60-12-8	
Phenoxyethyl isobutyrate	103-60-6	
Phenylisohexanol	55066-48-3	
Phenylpropanol	122-97-4	
Tetrahydrolinalool	78-69-3	
Tetrahydro-methyl-methylpropyl)-pyran-4-ol	63500-71-0	
Trimethylhexyl acetate	58430-94-7	
Trimethyl-propylcyclohexanepropanol (tmch)	70788-30-6	
gamma-Undecalactone	104-67-6	

Table 9-4: Not predictable.

Substance (INCI) name	CAS number	Structural alerts
Anisaldehyde	123-11-5	Due to insufficient /conflicting data; structural similarities to benzaldehyde suggest certain activity in man
Trichloromethyl phenyl carbonyl acetate	90-17-5	Due to insufficient /conflicting data
Methyl beta-naphthyl ether	93-04-9	Due to insufficient /conflicting data

9.1. General results

From this work with the included SAR predictions, the following observations can be made.

- For substances for which sufficient experimental/clinical evidence is missing, SAR analyses have been performed to predict a probable or possible risk of allergenic (sensitising) effect. These predictions are based on chemical reactivity and the recognition of structural features in a substance that are in common with the structural features that have been shown to cause sensitisation from other substances. In cases where the SAR analysis indicates a sensitisation potential, the substance should be investigated further to confirm or reject the conclusion drawn from the SAR analysis.
- Prediction of the sensitisation potential of compounds that can act via abiotic or metabolic activation (pre- or prohaptens) becomes more complex compared to that of compounds that act as direct haptens without any activation.
- The complexity of the prediction increases further for those compounds that can act both as prehaptens and prohaptens.
- Prediction of the sensitisation potential of compounds that can act as prehaptens is further complicated by the fact that the autoxidation patterns can differ due to differences in the stability of the intermediates formed, e.g. it has been shown that autoxidation of the structural isomers of linalool and geraniol results in different major haptens/allergens.

9.2. Conclusions

The SAR evaluation made in this section is based on *in cerebro* alerts applied by organic chemists.

- Applying only mechanism-based QSAR (QMM) as a tool in non-animal based risk assessment for skin sensitisation is of limited value for fragrance substances. This is due to major information gaps in the present model when addressing substances that act via abiotic or metabolic activation, and the high incidence of such substances in fragrances.
- Quantitative structure activity relationship (QSAR) models should be further developed, combining, as appropriate, information from *in silico*, *in chemico* and *in vitro* methods.
- SAR, as performed here, is only one consideration in the overall weight of evidence.

10. Exposure

Exposure to fragrance chemicals and other potential allergens is most commonly by direct skin contact. Exposures to fragrance chemicals occur from:

- Personal cosmetic use;
- Detergents and other household products;
- Medicaments;
- Occupation, i.e. personal hygiene, manufacturing ingredient(s), product in work process, plant materials;
- Secondary exposure from another individual (e.g. spouse, child);
- Toys;
- Oral intake;
- Airborne exposure.

Factors that are important for both the induction and elicitation of contact allergy are:

- Dose per unit area;
- Vehicle effects including penetration enhancers;
- Presence of skin irritants;
- Presence of other allergens (combination effects);
- Duration of skin exposure;
- Frequency of applications;
- Anatomical sites of exposure;
- Condition of the skin (barrier function impairment, pre-existing inflammation);
- Occlusion (e.g. in flexures, under clothing and personal protective equipment).

Fragrance mix ingredients are commonly present in cosmetic formulations (71, 222-224). Cosmetics based on natural ingredients may contain fragrance allergens at a higher concentration than other cosmetic products (225). The clinical significance of exposure to natural extracts is difficult to determine as there is often "hidden and variable" exposure to important and potent allergens in natural products.

10.1. Concentrations and quantities used

Consumers are exposed in daily life to fragrance chemicals from a large variety of products, such as cosmetics, toys, detergents and other cleaning products, etc. The fragrance exposure may be via dermal and/or inhalation route. With respect to "Terms of Reference" to the SCCS, only dermal exposure from cosmetics is addressed in this opinion. As cosmetics are the perfumed products most commonly used in daily life, potential fragrance allergens identified by the use of cosmetics also represent the exposures of these chemicals from other product categories. In recent years, it has become a trend to add fragrance chemicals to many other types of consumer products, such as children's toys, toilet paper and nappies, which may contribute significantly to the fragrance exposure of the consumer by the dermal route.

Factors for the fragrance exposure assessment by the dermal route require knowledge on:

- Product types (categorisation of scented products) used by the consumer.
- Market survey (impression of the qualitative and quantitative contents of different allergens in consumer products).

- Hydrolysis, metabolism or oxidation of a fragrance material, which may generate a potential skin allergen.
- Chemicals in the product matrix, which may significantly enhance or reduce dermal absorption of a fragrance material.

Fragrance materials, both defined chemical substances and natural mixtures of chemicals (essential oils), are used in all types of cosmetic products: perfumes, eau de cologne, eau de perfume (EDP), and eau de toilette (EDT), aftershave lotion, deodorants, skin care products, skin cleansers, make-up cosmetics, hair care products, and oral care products, etc. However, some unscented cosmetic products have also reached the market in the last decade. Products containing the highest concentration of fragrance chemicals are perfumes, followed by eau de cologne, eau de perfume (EDP) and eau de toilette (EDT). Concentrations of fragrance chemicals in deodorant products are lower than those in EDT/EDP products, but still significant. Aftershave products also contain relatively high amounts of fragrance chemicals. Other cosmetic products contain relatively low amounts, 0.1-1% of fragrance compound, compared to up to 30% fragrance compound in EDT/EDP (226). The fragrance compound are mixtures of 20 to over 200 synthetic fragrance chemicals or natural fragrance materials (essential oils), selected from over 3,000 fragrance materials (226). For the exposure assessment, levels of fragrance chemicals in cosmetics containing significant amounts of fragrance materials (i.e. EDP/EDT/aftershave/deodorant) should be selected. It may not be possible to detect/measure the amounts of all fragrance chemicals when present in highly diluted form in a cosmetic product such as skin care products, make-up cosmetics etc. On the other hand, if a fragrance is evaluated safe for use when present in significant amounts in a product, it will also be safe for use in other products. Also the analysis of trend of the use of individual fragrance materials should be based on monitoring their contents in fine perfumes and deodorants.

Ninety of the 100 fragrance materials used in annual volumes > 175 tons in perfume formulations are fragrances and the remaining ten are used for other functions such as solvents or antioxidants (IFRA, personal communication 2010).

Among the 26 fragrances currently requiring individual labelling, amyl cinnamal, benzyl benzoate, benzyl salicylate, butyl phenyl methyl propional, citral, citronellol, coumarin, eugenol, geraniol, hexyl cinnamal, hydroxyisohexyl 3-cyclohexene carboxyaldehyde (HICC), alpha-isomethyl ionone, and linalool are used in volumes greater than 175 ton. α -Amylcinnamyl alcohol, anisyl alcohol, benzyl alcohol, benzyl cinnamate, cinnamal, cinnamyl alcohol, farnesol, hydroxycitronellal, isoeugenol, *d*-limonene, methyl-2-octynoate, oak moss (*Evernia prunastri*), tree moss (*Evernia furfuracea*) are used in volumes less than 175 ton.

According to the information from the fragrance industry, 80% of the total fragrance chemical volume is used in cosmetics and 20% in household products.

Since the implementation of the regulation of labelling of 26 fragrance substances in cosmetic products, qualitative information on fragrance exposure from cosmetics is provided in some market surveys performed on cosmetics (Table 10-1, (227)) and (Table 10-2, (228)) and on consumer products including cosmetics (Table 10-3, (229); Table 10-4, (115); and Figure 10-1, (105)). Thus, the implementation of the regulation of fragrance allergens in detergents (Directive 648/2004/EC), similar to that for cosmetics, has also added to the knowledge of fragrance exposure to the consumer. These market surveys revealed that fragrance ingredients which are potent allergens and frequently cause allergies in consumers are used as ingredients in consumer products including cosmetics. The results of these surveys further revealed that limonene and linalool were the most commonly used fragrance chemicals in cosmetics, while anisyl alcohol, cinnamal, α -amylcinnamyl alcohol, oak moss and tree moss were the least used fragrance ingredients in cosmetics and other consumer products. In general, the most potent allergens were also the most infrequently used ingredients. Prior to the regulation of the 26 allergens, analysis of 21 selected fragrance chemicals in deodorants also revealed additional 66 potential allergens in these products on the basis of structure activity relationship (230).

Table 10-1: Presence in children's cosmetics of the 26 fragrance substances that are required to be labelled in cosmetics (227).

Fragrance substance		% Products labelled to contain the fragrance substance
INCI name	CAS number	
Amyl cinnamal	122-40-7	8.2
alpha-Amylcinnamyl alcohol	101-85-9	2.9
Anise alcohol	105-13-5	0
Benzyl alcohol	100-51-6	9.6
Benzyl benzoate	120-51-4	9.1
Benzyl cinnamate	103-41-3	2.9
Benzyl salicylate	118-58-1	9.6
Butyl phenyl methyl propional	80-54-6	7.7
Cinnamal	104-55-2	1
Cinnamyl alcohol	104-54-1	6.7
Citral	5392-40-5	8.2
Citronellol	106-22-9	10.5
Coumarin	91-64-5	4.8
Eugenol	97-53-0	7.2
Farnesol	4602-84-0	2.9
Geraniol	106-24-1	12
Hexyl cinnamal	101-86-0	10.1
Hydroxycitronellal	107-75-5	6.3
Hydroxyisohexyl-3-cyclohexene carboxyaldehyde	31906-04-4	5.8
Isoeugenol	97-54-1	0.5
Alpha-isomethyl ionone	127-51-5	5.8
<i>d</i> -Limonene	5989-27-5	23.1
Linalool	78-70-6	21.6
Methyl-2-octynoate	111-12-6	0
<i>Evernia prunastri</i> /oak moss	90028-68-5	0
<i>Evernia furfuracea</i> /tree moss	90028-67-4	0

Table 10-2: Usage trends in deodorants of fragrance chemicals that are required to be labelled in cosmetics.

Fragrance substance		88 products investigated in 2007 (228)			70 products investigated in 1998 (231)	
INCI name	CAS number	% Products labelled to contain the fragrance	Content in 23 selected products		Content in all 70 products	
			% Products found to contain the fragrance	Range(ppm)	% Products found to contain the fragrance	Range (ppm)
Amyl cinnamal [■]	122-40-7	10.2	17	2.3-165	31	1-617
alpha-amyl cinnamyl alcohol	101-85-9	-	-	-	n.a.	n.a.
Anise alcohol	105-13-5	2.3	9	1, 51	n.a.	n.a.
Benzyl alcohol	100-51-6	17.1	26	32-166	76	1-629*
Benzyl benzoate	120-51-4	25.0	48	3-4054	71	1-1075
Benzyl cinnamate	103-41-3	3.4	9	74, 143	n.a.	n.a.
Benzyl salicylate	118-58-1	39.8	48	136-5279	49	1-18758
Butyl phenyl methyl propional	80-54-6	48.9	70	1-5455	51	1-3732
Cinnamal [■]	104-55-2	1.1	4	5	17	1-424
Cinnamyl alcohol [■]	104-54-1	12.5	48	2-503	39	6-1169
Citral [□]	5392-40-5	26.1	44	39-554	n.a.	n.a.
Citronellol [□]	106-22-9	65.9	91	1-5848	81	1-5585
Coumarin [□]	91-64-5	33.0	52	3.8-1255	57	1-1411
Eugenol [■]	97-53-0	27.3	30	1-514	57	1-2355
Farnesol [□]	4602-84-0	14.8	39	9-1791	n.a.	n.a.
Geraniol [■]	106-24-1	48.9	87	1-399	76	1-1178

Opinion on fragrance allergens in cosmetic products

Fragrance substance		88 products investigated in 2007 (228)			70 products investigated in 1998 (231)	
Hexyl cinnamal [□]	101-86-0	33.0	48	1-4434	71	2-1684
Hydroxycitronellal [▪]	107-75-5	27.3	70	1-1746	50	1-1023
HICC [□]	31906-04-4	33.0	74	1-4431	53	1-1874
Isoeugenol [▪]	97-54-1	9.1	35	1-138	29	1-458
Alpha-isomethyl ionone	127-51-5	46.6	65	6-2588	61	1-2765
D-Limonene [°]	5989-27-5	53.4	70	1022-11386	n.a.	n.a.
Linalool [°]	78-70-6	53.4	96	8-3447	97	9-1927
Methyl-2-octynoat [°]	111-12-6	1.1	-	-	n.a.	n.a.
<i>Evernia prunastri</i> [▪] /oak moss	90028-68-5	4.6	n.a.	n.a.	n.a.	n.a.
<i>Evernia furfuracea</i> [▪] /tree moss	90028-67-4	2.3	n.a.	n.a.	n.a.	n.a.

Notes: HICC Hydroxyisohexyl-3-cyclohexene carboxyaldehyde.

- Fragrance not detected in any product.

n.a. Not analysed.

* Benzyl alcohol could not be determined in 49% of the products due to interference.

The most common fragrance allergens are contained in the two mixtures, which are used for diagnosing fragrance allergy, called Fragrance Mix I (▪) and Fragrance Mix II (°), besides the oxidation product of terpens (°), and tree moss extract are common allergens. Methyl-2-octynoate is an extreme, but rare allergen.

Opinion on fragrance allergens in cosmetic products

Table 10-3: Frequency of occurrence in consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (229).

INCI name of fragrance	PCP (n = 70)	MP (n = 59)	HP (n = 57)	WP (n = 44)	Cos (n = 39)	Deo (n = 17)	Dent (n = 14)	Total (n = 300)
Linalool	46	47	17	42	26	12	0	190 (63%)
Limonene	34	45	29	43	18	11	9	189 (63%)
Citronellol	23	24	21	37	25	15	0	145 (48%)
Geraniol	19	26	15	36	18	12	0	126 (42%)
BPMP	30	27	21	27	13	8	0	126 (42%)
Hexyl cinnamal	37	20	22	22	14	10	0	125 (42%)
Benzyl salicylate	23	23	10	31	15	12	0	114 (38%)
Alpha-isomethyl ionone	15	20	7	24	28	10	0	104 (35%)
Coumarin	12	27	8	23	12	8	0	90 (30%)
Lyr TM	17	24	3	24	15	5	0	88 (29%)
Eugenol	13	26	4	22	6	6	3	80 (27%)
Citral	2	28	6	29	7	2	0	74 (25%)
Benzyl benzoate	8	9	3	31	11	8	0	70 (23%)
Benzyl alcohol	9	8	1	30	9	3	1	61 (20%)
Hydroxycitronellal	5	6	1	30	6	4	0	52 (17%)
Isoeugenol	2	5	0	17	0	3	0	27 (9%)
Cinnamic alcohol	4	2	0	13	4	2	0	25 (8%)
Farnesol	1	3	0	17	2	0	0	23 (8%)
Amyl cinnamal	5	0	3	7	5	2	0	22 (7%)
Cinnamal	3	4	0	7	0	0	3	17 (6%)
Evermia prunastri/oak moss	0	3	0	5	5	0	0	13 (4%)
Benzyl cinnamate	2	0	0	8	0	0	0	10 (3%)
Evermia furfuracea/tree moss	1	5	0	3	0	0	0	9 (3%)
Anisyl alcohol	0	0	0	1	0	0	0	1 (0.3%)
Amyl cinnamic alcohol	0	0	0	0	0	0	0	0
Methyl heptine carbonate	0	0	0	0	0	0	0	0

INCI, International Nomenclature of Cosmetic Ingredients; PCP, personal care products; MP, men's products; HP, household products; WP, women's perfumes; Cos, cosmetics; Deo, deodorants; Dent, dental products; BPMP, butyl phenyl methyl propional; LyrTM, hydroxy-isohexyl-3-cyclohexene carboxaldehyde.

Table 10-4: Frequency in 516 consumer products of the 26 fragrance substances that are required to be labelled in cosmetics* (115).

Fragrance substance INCI name	% Product containing the chemical
D-Limonene	48.3
Linalool	35.8
Butyl phenyl methyl propional	24.8
Geraniol	22.1
Alpha-isomethyl ionone	21.7
Hexyl cinnamal	21.3
Citronellol	21.1
Benzyl salicylate	18.6
Coumarin	17.0
Eugenol	15.7
Benzyl alcohol	15.3
Benzyl benzoate	14.7
Hydroxyisohexyl-3-cyclohexene carboxyaldehyde	12.8

Fragrance substance INCI name	% Product containing the chemical
Citral	11.6
Hydroxycitronellal	10.8
Amyl Cinnamal	7.9
Anise alcohol	7.0
Cinnamyl alcohol	6.4
Farnesol	3.9
Isoeugenol	3.1
Cinnamal	2.5
Benzyl cinnamate	2.3
Amylcinnamyl alcohol	1.9
Methyl-2-octynoate	1.0
<i>Evernia prunastri</i> •/oak moss	0.8
<i>Evernia furfuracea</i> •/tree moss	0.4

Note: * Consumer Products: Cosmetics and household products with labelling of the 26 fragrance allergens. The content of these fragrances was confirmed by chemical analysis.

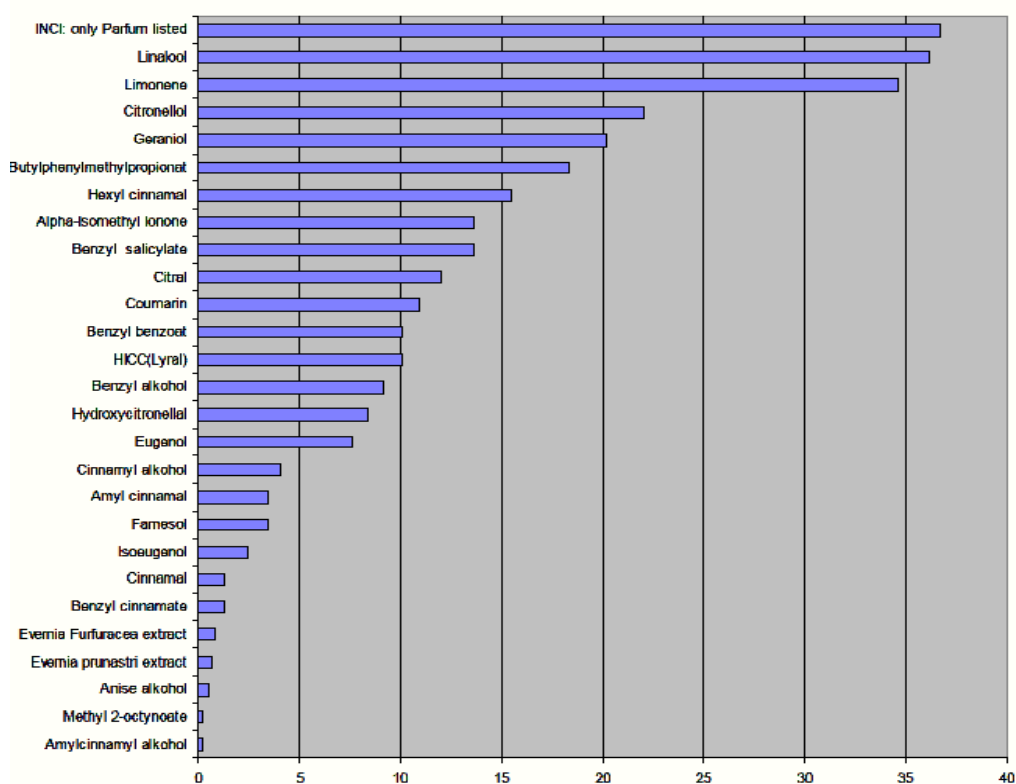


Figure 10-1: Frequency of occurrence in 3,000 consumer products of the 26 fragrance allergens that are required to be labelled in cosmetics and detergents (CVUA Karlsruhe, Germany, 2006/2007), according to (105).

Contents of fragrance substances determined in cosmetic products have been described in several studies, both before and after the regulation of the 26 fragrance allergens. The studies prior to the regulation of the 26 fragrance allergens included many, but not all of these 26 allergens. On the other hand, these studies included some other possible fragrance allergens. The quantitative analysis of fragrance substances has been performed in prestige perfumes (5, 157, 232-234), deodorants (228, 231), children's cosmetics and cosmetic toys (115, 227, 235), products marketed as natural cosmetics (225) and in cosmetics used by patients with contact allergy to fragranced products (35, 71). Quantitative analyses have revealed that the consumer is exposed to most, but not all of the 26 fragrance allergens from the use of cosmetics. However, when fragrance exposure from other consumer products, for example detergents and other household products is also taken into consideration (Table 10-3, Table 10-4, Figure 10-1), (105, 115, 229, 236), exposure to all of the 26 allergens is foreseeable in daily life. Although from the data available, the exposure to α -amylcinnamyl alcohol, cinnamal, methyl-2-octynoate, *Evernia prunastri* (oak moss) and tree moss may appear to be low, these are very strong allergens.

The changes in the use of fragrance chemicals in cosmetic formulations, during last 12 years, i.e. before and after the regulation of the 26 fragrance allergens, is reflected in the studies concerning contents of fragrances substances in popular perfumes (5, 232). As described in Table 10-5, the content of FM I allergens in prestige perfumes was significantly reduced from 1996 to 2003. Whether this is also the case for the perfumes sold as natural cosmetics (Table 10-6) has not yet been investigated.

Table 10-5: Concentration of Fragrance Mix I ingredients in five prestige perfumes before and after the regulation of the 26 fragrance allergens.

Fragrance INCI name	Concentration in the perfumes before regulation (5)			Concentration in the perfumes after regulation (232)		
	In no. of perfumes	Range % (w/w)	Mean % (w/w)	In no. of perfumes	Range % (w/w)	Mean % (w/w)
Geraniol*	5	0.072- 0.432	0.340	5	0.090- 0.236	0.156
Cinnamal	2	0.002- 0.002	0.002	0	-	-
Hydroxy- citronellal	5	0.222- 0.979	0.615	5	0.015- 0.478	0.169
Cinnamyl alcohol	4	0.068- 0.232	0.147	0	-	-
Eugenol	5	0.032- 0.738	0.337	2	0.001, 0.001	0.001
Isoeugenol	3	0.026- 0.249	0.119	2	0.001, 0.004	0.003
Amyl cinnamal	1	0.019	0.019	0	-	-

Note: * Due to interference by linalyl acetate, concentration of geraniol+linalyl acetate is reported.

Table 10-6: Concentrations of Fragrance Mix I ingredients, hexyl cinnamal and coumarin in 22 perfumes marketed as natural cosmetics investigated in 1996.

Fragrance	In no. of perfumes	Concentration % (w/w)
Geraniol	14	1.191*
Cinnamal	3	0.089, 0.109, 2.101
Hydroxycitronellal	5	0.135-6.044
Cinnamyl alcohol	8	0.035-2.289
Eugenol	2	0.027, 0.139
Isoeugenol	8	0.194-3.039
Amyl cinnamal	9	0.105-7.706
Coumarin	11	0.046-6.043

Note: * Quantification was performed in one sample only, due to interference by a very large amount of linalyl acetate in other samples.

The trend in the use of most of the fragrance allergens in deodorants before and after their regulation is reflected by the two studies performed by Rastogi et al. (228, 231). The results of these studies cannot be directly compared, because the study from 1998 included randomly selected deodorants, while selection of the deodorants for the 2007 study was based on the labelling of the presence of known strong fragrance allergens in these products. The number of products analysed in the 1998 study were three times more than those analysed in 2007, but not all of the 26 fragrance allergens were analysed in the 1997 study. However, an indication of the change in the use of the fragrance allergens during 1998-2007 may be obtained by reviewing the results of these two studies. Among the 17 common fragrance substances studied in the two studies, the frequency of use of 16 of these substances in deodorants was reduced in 2007 compared to that in 1998 (Table 10-2). The frequency of use of butyl phenyl methyl propional in deodorants appeared to be unchanged. The contents of benzyl alcohol, benzyl salicylate, cinnamal, cinnamyl alcohol, eugenol, geraniol, isoeugenol and linalool were found to be lower in the deodorants analysed in 2007 compared to those in 1998. Citronellol, coumarin and alpha-isomethylionone contents in the deodorants were similar in both studies, but concentrations of benzyl benzoate, butyl phenyl methyl propional, hexyl cinnamal, hydroxyisohexyl-3-cyclohexene carboxyaldehyde and linalool were much higher in deodorants in 2007 compared to those in 1998. This analysis of trend of use of fragrance allergens in cosmetic products indicates that the regulated fragrance allergens are used less frequently, but exposures from some of the regulated fragrance allergens may be much higher compared to those before regulation.

Table 10-7: Atranol and chloroatranol content in eau de toilette/eau de perfume, investigated in 2004 and in 2007.

	2007 Study	2004 Study
No. of samples	22	17
Atranol present in no. of samples	15 (68%)	12 (70%)
Atranol content	ppb (ng/ml)	ppb (ng/ml)
Range	n.d.-880	n.d.-791
Mean±SD	157±249	97±224
Median	47	20
Chloroatranol present in no. of samples	9 (41%)*	14 (82%)
Atranol content	ppb (ng/ml)	Ppb (ng/ml)
Range	0.9-208	1-175
Mean±SD	63±73	36±51
Median	22	10

Notes: n.d. Not detected.

* $P < 0.05$ (chi-square test).

SD: Standard deviation.

Atranol (CAS no. 526-37-4) and chloroatranol (CAS no. 57074-21-2), constituents of oak moss and tree moss have been shown to be very potent fragrance allergens (237, 238). The EC Scientific Committee on Consumer Products (SCCP) recommended that atranol and chloroatranol should not be present in cosmetic products (239). Two other commonly used fragrance chemicals, isoeugenol (240) and hydroxyisohexyl-3-cyclohexene carboxyaldehyde (HICC) (71), have also been shown to be important contact allergens. The contents of atranol, chloroatranol, isoeugenol and hydroxyisohexyl-3-cyclohexene carboxyaldehyde in fine fragrances was determined for the exposure assessment of these fragrances (233). The results revealed that isoeugenol was present in 56%, HICC in 72%, atranol in 59%, and chloroatranol in 36% of the 22 eau de toilette/eau de parfum products. The concentrations of isoeugenol were, in all products, below 0.02% which is the maximum concentration recommended by the fragrance industry. HICC reached a maximum concentration of 0.2%, which is 10-fold higher than the maximum tolerable concentration considered safe by the EC Scientific Committee (241). The concentrations of atranol and chloroatranol in the products investigated in 2007 were comparable to those found in similar products in 2004 (Table 10-7, (233, 234). A significant decrease in the frequency of the presence of chloroatranol in the products was found in 2007 (Table 10-7).

10.2. Global exposure (household and occupational exposures)

Fragrances are used in cosmetics that the consumer applies to themselves, as described in the previous section. In addition, exposure to fragrance substances is possible by a number of other exposure routes briefly outlined in this section.

Topical pharmaceutical products

In a study from Belgium, 370 of the 3,280 topical products marketed in Belgium have been found to contain one or more of 66 fragrance substances (242). This publication also contains a description of causative fragrance allergens in 127 patients reacting to 48 specific topical products. In a broader sense, exposure of the patient by extracts used in aromatherapy falls in this category as well.

Childrens products and toys

Children's products may contain fragrance allergens and high levels may be present (235). It has been stated that children may become sensitised to fragrance chemicals used by their mothers (243).

Clothing

Washed fabrics have been reported to contain fragrances (244). Odour-neutralising agents are sometimes used for shoe insoles. In one case, an insole containing cinnamon, has been reported to lead to plantar vesicular contact dermatitis due to contact sensitisation to FM I and, in the breakdown, to cinnamal and cinnamyl alcohol (245).

Cleaning agents and other household products

Contact dermatitis from geraniol in washing-up liquid has been reported (246). Terpenes are used as solvents and cleansing agents (e.g. limonene) (247) and have been reported as cause of hand dermatitis (248, 249). In an analysis of 59 household products the most common fragrance allergens were limonene (78%), linalool (61%) and citronellol (47%) (250). In a review of 301 cosmetic and detergent consumer products in Sweden, in half of the cosmetics and one-third of the detergents, one or more of the 26 fragrances requiring labelling were identified (251). In the UK, a review of 300 consumer products showed that linalool and limonene were present in 63% of products. Dental products contained on average 1.1 fragrance substances that are presently required to be labelled and women's perfumes contained 12 of these fragrance substances (Table 4-1 and Table 4-3) (229).

Candles

The dermal hand transfer of three fragrance materials (cinnamic aldehyde, d-limonene and eugenol) from scented candles was determined in ten subjects (i.e. 20 hands) after grasping scented candles for five consecutive 20 second exposures/grasps. The total mean residues of cinnamal and eugenol transferred per grasp from the candles to the hands were 0.255 µg/cm(2) and 0.279 µg/cm(2), respectively (252).

Food

Food causing cheilitis or bullous stomatitis (e.g. due to cinnamal (253)) or lichen planus-like lesions (e.g. due to cinnamal (254)) or contact gingivitis (e.g. due to eugenol (255)) has been reported. Moreover, food containing fragrance allergens, e.g. citrus oil terpenes (256) may cause allergic contact dermatitis by handling this food.

Occupational exposure

In a number of occupations, contact allergy to fragrances is more common than in others, including geriatric nurses, masseurs and physiotherapists, metal furnace operators and potters/glass makers, according to a multifactorial analysis (90). Moreover, hairdressers, beauty therapists and aroma therapists are examples of occupations where there is occupational exposure to fragrance-containing cosmetic and other products. Cleaners are exposed to fragrance-containing household products (e.g. detergents). Cooks and bakers are exposed to flavour chemicals and spices. Healthcare workers are also at risk of acquiring fragrance contact allergy. "Odour maskers" may contain important fragrance allergens (89, 90, 257-259). Occupational exposure and

occupational ACD to fragrances have been described in perfume bottlers (260). Industrial use of a powder masking the vinyl smell of car seats, containing cinnamal, causing occupational ACD has been reported (259).

A number of fragrance chemicals are also used as biocides (see Commission Regulation (EC) No 1451/2007 of 4 December 2007 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market, published 11.12.2007 EN Official Journal of the European Union L 325/3 –L325/65), see Table 10-8 below.

Table 10-8: Parts of Annex I to (EC) No 1451/2007 (see above): "Active substances identified as existing", if use is 'perfuming' or 'masking' according to CosIng.

Biocide	EINECS	CAS number	Biocidal product group
Linalool	201-134-4	78-70-6	19
Geraniol	203-377-1	106-24-1	18, 19
Benzyl benzoate	204-402-9	120-51-4	2, 18
Eugenol	202-589-1	97-53-0	Not given
Farnesol	225-004-1	4602-84-0	Not given
(R)-p-mentha-1,8-diene	227-813-5	5989-27-5	12
Citriodiol/mixture of cis- and trans-p-menthane-3,8 diol	255-953-7	42822-86-6	1, 2, 19
Citral	226-394-6	5392-40-5	Not given
Pine ext.	304-455-9	94266-48-5	10
TANACETUM CINERARIIFOLIUM FLOWER EXTRACT	289-699-3	89997-63-7	18
Citrus oils (main component: limonene)	several	various	
Clove oil (main component: eugenol (83.8 %), caryophyllene (12.4 %))	/	8000-34-8	

Product groups(According to Biocide Directive 98/8/EC)

- 1 Human hygiene biocidal products
- 2 Private area and public health area disinfectants and other biocidal products
- 3 Veterinary hygiene biocidal products
- 10 Masonry preservatives
- 12 Slimicides
- 18 Insecticides, acaricides and products to control other arthropods
- 19 Repellents and attractants

The above illustrates that the consumer is exposed to fragrance substances from a wide variety of cosmetic products, other consumer products, pharmaceuticals and occupational exposures.

All these exposures are of importance in the context of contact allergy as it is not the source of exposure that is critical for both induction and elicitation, but the cumulative dose per unit area.

10.3. Exposures related to particular anatomical sites

Contact allergy to fragrances most often causes dermatitis of the hands, face and axillae. Axillary involvement has been shown to be statistically related to fragrance allergy (9). It is recognised that the axillary skin is a problematic area as it is moist, occluded and is easily irritated. Moreover, facial eczema is a common manifestation of fragrance allergy (3, 47). There is an association between fragrance allergy and hand eczema or aggravation of hand eczema (13-15). Vehicles may influence elicitation capacity of an allergen and the presence of detergents (surfactants) as in hand cleaning products may increase the clinical response by a factor of 4-6 (261). Men using wet shaving as opposed to electric razors have an increased risk of being fragrance allergic (17), most likely due to microtraumata and to the presence of surface active substances in shaving foam.

In use tests, the upper arm has been shown to be more sensitive than the forehead and lower arm (262). The axillae, neck and face are more sensitive than the upper arms (10). The threshold of elicitation may vary depending on the volatility of the substance (263). A cumulative effect of exposures occurs so that repeating exposures cause elicitation in more individuals (264).

Patients appear to become sensitised to fragrances primarily from deodorants and perfumes and to a lesser extent from other cosmetic types (74). Allergic contact dermatitis may develop where a perfume has been applied (behind ears, neck, upper chest, antecubital fossae, wrists and the axillae bilaterally (265). Following this, eczema may appear, or be worsened by, the use of a variety of product types including other cosmetics, household products, industrial products and flavours.

The association between contact allergy to fragrance ingredients and certain anatomical sites, which mirrors exposure to fragrance-containing products on these anatomical sites, has been described in several publications (266, 267), see above. However, due to the potential confounding effect of other factors, at least on some anatomical sites, an adjusted analysis will provide a more valid impression of the association between certain anatomical sites and contact allergy to fragrance ingredients. As an adjusted, multifactorial analysis relies on: (i) a substantial number of observations (patients tested); and (ii) an outcome prevalence not too close to 0%, such an approach has, hitherto, been limited to FM I.

In a paper published 2001, data from the IVDK in terms of patch test reactions to FM I and relevant clinical and demographic information of the patients tested (n=57,779) was studied by Poisson regression analysis (90). Risk was quantified by the prevalence ratio, which can be interpreted as an estimate of relative risk, i.e. the factor by which the risk of being sensitised to FM I (in this example) is to be multiplied ($RR > 1$: elevated risk; or $RR < 1$: reduced risk) if a certain "risk factor" is present, compared to those patients in whom this risk factor is not present (the reference category) (general aspects of such analyses are discussed in (268)). In the analysis, potential risk factors and confounders, respectively, including occupation, year of patch testing (to address a possible time trend), sex, age, past or current atopic dermatitis, in addition to anatomical site. The relevant part of Table 3 of (90) is reproduced below.

Table 10-9: Result of a Poisson regression analysis of patients tested with the Fragrance Mix between January 1992 and December 1998, considering two alternative outcomes – part I: non-occupational factors

Attribute	Prevalence (%)	At least + (11.5%)		At least ++ (4.0%)	
		PR	95% CI	PR	95% CI
Age:					
≤30	26.7	1.00	Reference	1.00	Reference
>30–44	23.8	1.42	1.31 to 1.53	1.61	1.40 to 1.84
>44–58	25.6	1.67	1.55 to 1.80	1.90	1.66 to 2.16
>58	23.9	1.93	1.77 to 2.10	2.07	1.79 to 2.39
Sex (female)	64.5	1.29	1.21 to 1.37	1.18	1.07 to 1.31
Main site:*					
Trunk	2.9	1.00	Reference	1.00	Reference
Hands	29.9	1.24	1.07 to 1.46	1.28	0.98 to 1.67
Arm	3.8	1.23	1.01 to 1.49	1.19	0.86 to 1.65
Face	15.2	1.20	1.03 to 1.42	1.13	0.86 to 1.48
Neck	1.4	1.39	1.10 to 1.75	1.31	0.88 to 1.94
Feet	2.8	1.26	1.02 to 1.55	1.19	0.84 to 1.68
Leg	8.7	1.59	1.36 to 1.89	1.50	1.14 to 1.99
Axilla	0.9	2.77	2.20 to 3.46	2.73	1.87 to 4.00
Other site	8.9	0.66	0.55 to 0.80	0.48	0.35 to 0.67

*Additionally controlled for several more sites—none of these associated with a significantly increased or decreased risk.

Compared to the trunk, which was arbitrarily chosen as the reference category, all other anatomical sites are associated with an increased risk of being sensitised to FM I (significantly if the lower limit of 95% CI is > 1). Most evidently, dermatitis of the axilla(e) is strongly associated with contact allergy to FM I, presumably due to the application of deodorants. Furthermore, the part of the table shown above illustrates a strong, positive age gradient, i.e. the older patients are, the more likely they are to be sensitised to FM I, the risk being almost double when comparing the oldest with the youngest age group. This observation is in concordance with a bivariate (unadjusted) association between age and contact allergy to FM I found in another study (89). This association is presumably the result of life long exposures and cumulative risk.

In a similar analysis of *Myroxylon pereirae* resin, published in 2002 (269): (i) an even stronger age gradient; and (ii) no particular association to axillary dermatitis (included in the “other” category) was found (Table 10-10).

Table 10-10: Association between selected risk factors and positive patch test to *Myroxylon pereirae* resin. For full model see (269). Risk quantified with the prevalence ratio (PR) with accompanying 95% confidence interval (CI).

Factor	PR	95% CI
Atopic dermatitis, past or present	1.02	(0.95-1.10)
Female sex	1.13	(1.06-1.20)
Site		
Trunk	1.00	(reference)
Hand or Arm	1.03	(0.94-1.12)
Foot or Leg	1.76	(1.61-1.92)
Head or Neck	0.94	(0.86-1.03)
“Other” site	0.72	(0.64-0.81)
Missing site	1.07	(0.97-1.19)
Age		
30 years and younger	1.00	(reference)
31 to 44	1.92	(1.73-2.12)
45 to 58	2.87	(2.61-3.16)
58 or older	3.85	(3.49-4.25)

10.4. Conclusion

There are various modes of exposure to fragrances, including not only products used for their scent, such as perfumes and eau de toilette, after shaves, and deodorants, but also types of products where scent is an added feature, such as other cosmetic categories (including wipes), topical pharmaceuticals, household products, and products encountered in the occupational setting.

Consumer exposure can change over time, both qualitatively and quantitatively.

Different routes of exposure are reflected by certain anatomical sites affected: deodorants are associated with axillary dermatitis, the axillary skin being particularly vulnerable to sensitisation due to occlusion, maceration and irritation. However, while sensitisation and initial disease may follow a distinct pattern, later less specific exposures, e.g. via hand creams, cleaning lotions etc. may be sufficient to cause allergic contact dermatitis.

11. Dose-response relationships and thresholds

The dose-response relationship between exposure to contact allergens and induction of allergy, i.e. sensitisation, is well established in animal models and by experiments in healthy volunteers (270). It seems that not only the dose per unit area of allergen (271), but also the number of exposures, i.e. the accumulated dose, is of importance for the risk of induction of contact allergy (272). The induction of contact allergy is an immunological process (type IV-allergy), which is without any clinical symptoms. In the case of continued exposure or re-exposure with a sufficient dose of allergen, elicitation will occur. Elicitation is an inflammatory response (eczema) with clinical symptoms of erythema, induration and in some cases vesicles. Studies of the elicitation response are normally done in patients with an allergy to the substance in question. Different provocation models exist (see chapter 11.2.1). Elicitation experiments in healthy human volunteers following the induction have only rarely been performed (273) and may be considered a less valid model than patient studies. The reason is that following experimental induction, the level of sensitivity may not be at the same level as in a real life situation and that individuals who have actually acquired the disease are a more relevant endpoint to study.

Knowledge of the dose-response relationship provides an opportunity to establish levels of exposure which are safe for the majority of individuals. In the following chapter, the use of different data and models for the establishment of such safe levels in relation to fragrance ingredients are explored. The focus will be on those chemicals, which have been identified in chapter 7.1 as established contact allergens in humans and which have already given rise to a significant number of published cases (category 3 or more): cinnamal, cinnamyl alcohol, citral, coumarin, eugenol, farnesol, geraniol, hydroxycitronellal, isoeugenol. Limonene and linalool are considered in chapter 5 as their ability to cause sensitisation depends on air oxidation, and hydroxyisohexyl 3-cyclohexene carboxaldehyde is considered in chapter 4.3.2 and 11.4.

11.1. Induction

A model for dermal sensitisation quantitative risk assessment (QRA) has been developed and implemented by the fragrance industry. This model relies on thresholds, no effect or low-effect levels, established in healthy human volunteers and/or in animal experiments, mainly the local lymph node assay (LLNA) (see chapter 8.1). A set of safety factors are applied for inter-individual differences, for vehicle effects and for use considerations, stated to give rise to a safety margin from 10 to 1000 (274). In this way, a so-called "acceptable exposure level" is derived. The exposure to an allergen in different types of products should be below this level. The restrictions, which have been introduced by the fragrance industry based on the QRA model, are given in Table 11-1 for some important product categories.

The IFRA guidelines give concentration limits for 11 product categories (http://www.ifraorg.org/en-us/standards_1, last accessed 2011-11-02), three of which are mentioned in Table 11-1. These three products have the lowest concentrations except for lip products, which give a slightly lower concentration limit.

Table 11-1: Current IFRA restrictions based on induction experiments.

Fragrance chemicals	IFRA guideline ¹		
	Deodorant (%)	Hand cream (%)	Perfume (%)
Cinnamal	0.02	0.05	0.05
Cinnamyl alcohol	0.1	0.4	0.4
Citral	0.05	0.3	0.6
Coumarin	0.13	0.8	1.6
Eugenol	0.2	0.5	0.5
Farnesol	0.11	0.6	1.2
Geraniol	0.4	2.8	5.3
Hydroxycitronellal ²	0.2	1.0	1.0
Isoeugenol ²	0.01	0.02	0.02

Notes: 1) Exposure per mg/cm²/day is based on 8.5 mg/cm²/day for deodorants, 2.2 for perfumes and 4.2 for hand creams as it is these exposure levels that are used by the IFRA.
 2) Cosmetic Directive Annex III: Hydroxycitronellal restricted to 1% in all products and isoeugenol to 0.02% in all products.

The SCCP evaluated this methodology (275) as well as its application to three model fragrance substances.

It was, among other things, concluded that:

“The data provided show that the application of the dermal sensitisation QRA approach would allow increased exposures to allergens already known to cause allergic contact dermatitis in consumers. The model has not been validated and no strategy of validation has been suggested. There is no confidence that the levels of skin sensitisers identified by the dermal sensitisation QRA are safe for the consumer.”

and that:

“Identification of safe levels of exposure to existing substances known to cause allergic contact dermatitis in the consumer should be based on clinical data and/or elicitation low-effect levels. Currently, these are the only methods which have proven efficient in reducing/preventing existing problems of sensitisation/allergic contact dermatitis in the consumer.”

11.2. Elicitation

11.2.1. General considerations

A response in terms of elicitation of allergic contact dermatitis by application of the (suspected) allergen under standardised conditions is the outcome of interest of the routine diagnostic procedure for suspected contact allergy, the patch test. While the patch test procedure is largely standardised, it is optimised as a diagnostic tool for contact allergy. Thus exposure conditions are not comparable to actual exposures occurring in the daily life or working environment of the patient, which often involve long-term, repeated and low-dose contact with the allergen. Here, procedures such as the repeated open application test (ROAT) or provocative use test are often used, because they reflect actual exposure much better and can be used, for instance, to validate the current clinical relevance of a positive PT reaction.

Generally, exposure of a sensitised patient to a set of graded doses (quantity/area) of the suspected allergen, i.e. threshold testing, will allow not only quantitative diagnosis of the presence or absence of specific contact sensitisation but will additionally provide evidence on the intensity (degree) of sensitisation. This may have important individual consequences in terms of everyday or occupational exposures being capable (or not) of eliciting allergic contact dermatitis. However, beyond the individual perspective, clinical dose-response data collected from sensitised individuals provide a valuable estimate of the usual doses/unit area resulting in a positive, allergic response in a certain proportion of sensitised persons, e.g. 10, 50 or 90%. Maximum concentration levels can be derived, which are safe in terms of eliciting allergic reactions in only a defined low percentage of sensitised persons. As such data will always be based on small samples, the precision of the estimate should be considered, and therefore results are preferably given with confidence intervals.

A statistically significant relationship between threshold concentrations in the ROAT and patch test has been found, on analysing results from different allergens (see Table 11-2) (276), but the dose of allergen per unit area per application needed to elicit a reaction in the two study methods is not the same. A translation factor between the two methods has been suggested for non-volatile substances: $ED_{xx}(ROAT) = 0.0296 * ED_{xx}(\text{patch test})$ based on testing nickel and methyldibromo glutaronitrile (276). Based on this the eliciting dose per application in an open test is 33 times lower than in the patch test. In practice it means that the cumulative dose in a ROAT (in $\mu\text{g}/\text{cm}^2$) in two weeks with two applications per day (total 28 applications) will be almost identical to the eliciting patch test dose (in $\mu\text{g}/\text{cm}^2$) for a given number of responders (see Figure 11-1). For a given cut-off point the elicitation dose determined by patch testing will be higher than determined by ROATs.

Table 11-2: Spearman's rank correlation between the threshold concentration in the patch test and the repeated open application test for three allergens.

Allergen	Number of patients	Correlation coefficient	P-value
Nickel	18	0.45	0.033
MDBGN	15	0.76	0.0021
HICC	16	0.59	0.011

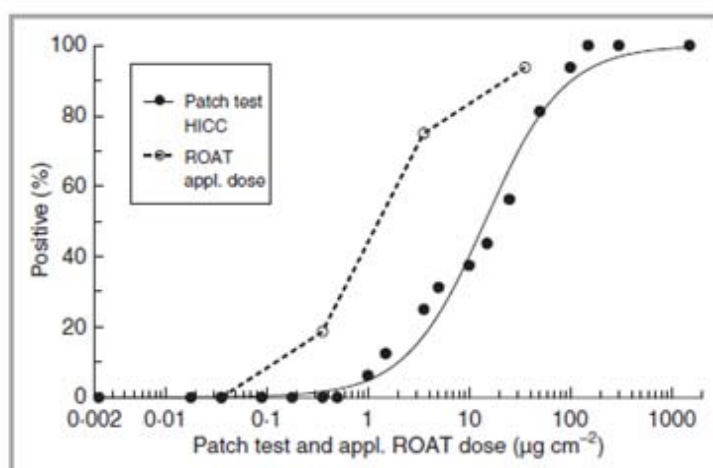


Figure 11-1: The fitted dose-response curve for patch test (solid line) is seen to be displaced to the right compared to the observed response from repeated open applications of the same allergen (HICC). It means that a smaller dose per application is needed to elicit a response than by one single occluded application as in the patch test.

In the translation between methods, evaporation needs to be taken into consideration for volatile substances. The experience, based on a study of the fragrance ingredient HICC and using the results from the literature on isoeugenol, is that if the same equation is used as for non-volatile substances, the response in the ROAT will be overestimated by a factor 3 to 4. Thus, the translation factor would be 0.1060 instead of 0.0296, but this needs to be confirmed by other fragrance allergens. This implies that for the fragrance ingredients tested, the eliciting dose per application in a ROAT was 9.4 times lower than the patch test compared to a 33 times lower dose for non-volatile substances (276). This needs to be confirmed by studying other fragrance allergens. Thus, according to these experiments, the dose ($\mu\text{g}/\text{cm}^2$) eliciting a response in threshold patch testing will be at most 33 times higher than established in the ROAT if an identical vehicle is used.

Volatility effects in skin sensitisation

The potency of volatile skin sensitisers can be underestimated, to an extent depending on how rapidly it evaporates, by assays such as the LLNA in which the test substance is applied topically to exposed healthy skin without occlusion. Such sensitisers present a greater sensitisation risk to consumers when the skin is occluded by clothing and/or compromised, than when healthy non-occluded skin is exposed.

Volatility at physiological temperature, say 40°C , is represented by the vapour pressure p_{40} at that temperature. This is related to the boiling point T_B by the Clapeyron-Clausius equation, which can be written (277):

$$\text{Log}(p_{40}) = - (T_B - 40)\text{Tr}/2.303RT$$

Where p is in atmospheres, T_B is in $^\circ\text{C}$, R is the gas constant, Tr is the Trouton constant (also defined as the molar entropy of vaporisation, and equal to $22 \text{ cal}\cdot\text{deg}^{-1}$ for many organic compounds) and T is physiological temperature in degrees absolute ($= 313$ for 40°C).

It has been shown, in experiments where evaporation from a glass slide is measured under simulated LLNA conditions, that 2-hexenal ($T_B = 146\text{--}149^\circ\text{C}$, $p_{40} = 17 \text{ mmHg}$) evaporates rapidly, less than 20% remaining after 5 minutes, whereas with cinnamal ($T_B = 248^\circ\text{C}$, $p_{40} = 0.5 \text{ mmHg}$), more than 90% remains after 1 hour (278). In agreement with these findings, cinnamal fits a QSAR relating LLNA EC3 to reactivity, whereas the EC3 for 2-hexenal is higher (lower potency) than predicted from its reactivity.

The above is only a partial rationalisation, since different solubilities in different vehicles will influence the tendency to evaporate, according to Henry's law.

11.2.2. Studies on specific fragrance ingredients

Studies concerning chloroatranol/atranol, cinnamal, hydroxycitronellal, hydroxyisohexyl 3-cyclohexenecarboxaldehyde and isoeugenol have been identified. These are summarised in Annex III.

Overview of results

In four studies dummy deodorants spiked with a single fragrance allergen in realistic use concentrations have been used to study elicitation responses, unscented deodorants were used as control products in paired designs. The deodorants were used by patients sensitised to the fragrance allergen in question as well as a healthy control group

(without fragrance allergy) (102,103,104,279). Between 76 and 100% of the sensitised individuals reacted to the deodorants spiked with allergen, isoeugenol, cinnamal, hydroxycitronellal and hydroxyisohexyl 3-cyclohexene carboxaldehyde, and none of the controls (Table 11-4).

Table 11-3: Overview of results of deodorant provocation investigations with different allergens. Frequency in % of test groups, which reacted at different doses of allergen applied in a roll-on deodorant in the axilla, is given in the table.

Dose in ppm in deodorant	Isoeugenol	Cinnamal (1)	Cinnamal (2)	Hydroxycitronellal	HICC
0	0	0	0	0	0
63	23				
100			11		
200	69				64
320		25	55	57	
600					85
630	76				
1000		75	88	71	
1800					100
3200		100		100	
No. test persons	13	8	9	7	14
No. of control persons	10	20		7	10
% control persons, who reacted	0	0		0	0
Exposure according to study should be:	< 63 ppm	<100 ppm		<320 ppm	< 200 ppm
Reference	(279)	(103)		(104)	(102)

Note: HICC hydroxyisohexyl 3-cyclohexene carboxaldehyde.

Eleven studies concerning dose-response results of the five allergens listed above were identified, including the above mentioned studies of deodorants. An overview of the results of the studies concerning thresholds is given in Table 11-4. In Annex III the details of each study are given.

Table 11-4: Overview of threshold results from clinical studies.

“Observed” means that the proportion was actually observed in the study while “estimated” means that the value is derived from a fitted curve, i.e. is interpolated.

Chloroatranol			
ROAT			Ref.
In ethanol 92 % positive	0.025 µg/cm ²	observed	(238)
In ethanol 100% positive	0.125 µg/cm ²	observed	(238)
PATCH TEST			

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ED10%	0.0004 µg/cm ²	estimated	(238)
ED50%	0.0045 µg/cm ²	estimated	(238)
Cinnamal			
ROAT			
In ethanol no effect	0.02%	observed	(101)
In ethanol 44 % positive	0.1%	observed	(101)
In ethanol 72 % positive	0.8%	observed	(101)
Deodorant matrix 11% positive	0.26 µg/cm ² (0.01%)	observed	(103)
Deodorant matrix 41% positive	0.84 µg/cm ² (0.032%)	observed	(103)
Deodorant matrix 82% positive	2.63 µg/cm ² (0.1%)	observed	(103)
PATCH TEST			
ED50%	96 µg/cm ²	estimated	(101)
No effect level	0.4 µg/cm ² (0.01%)	observed	(101)
No effect level	NG (0.002%)	observed	(103)
HICC			
ROAT			
In a cream base ED10%	4.9 µg/cm ²	interpolated	(105)
In a perfume (ethanol) ED10%	1.2 µg/cm ²	interpolated	(105)
In ethanol 61% positive	15.3 µg/cm ² (3.4-22.2)	observed	(224)
In ethanol 89% positive	126.2 µg/cm ² (40.5-226.2)	observed	(224)
In ethanol/water no response	0.0357 µg/cm ²	observed	(263)
In ethanol/water ED10%	0.064 µg/cm ²	estimated	(263)
In deodorant matrix between 64% to 100% positive	0.79 µg/cm ² (median)	observed	(102)
PATCH TEST			
ED10% (95% CI)	0.662 µg/cm ² (0.052-2.35)	estimated	(263)
ED10%	0.75 µg/cm ²	estimated	(102)
ED10%	0.9 µg/cm ² 29 (7-69) ppm	estimated	(224)
ED50% (95% CI)	11.1 µg/cm ² (3.41- 33.1)	estimated	(263)
ED50% (95% CI)	18.3 µg/cm ² (3.41- 33.1)	estimated	(102)
ED50% (95% CI)	20 µg/cm ² 662 (350-1250) ppm	estimated	(224)
No effect level	<0.0022 µg/cm ²	observed	(263)
Hydroxycitronellal			
ROAT			
Deodorant matrix 57 % positive	0.94 µg/cm ² (0.032%)	observed	(104)
Deodorant matrix 71 % positive	2.94 µg/cm ² (0.1%)	observed	(104)
Deodorant matrix 100 % positive	9.40 µg/cm ² (0.32%)	observed	(104)
PATCH TEST			

No effect level	<0.00012 % (=0.036 µg/cm ²)* (*calculated)	observed	(104)
Isoeugenol			
ROAT			
in ethanol 63% positive	5.6 µg/cm ²	observed	(100)
in ethanol 42% positive	2.2 µg/cm ²	observed	(264)
in ethanol 67% positive	9.0 µg/cm ²	observed	(264)
Deodorant matrix 23 % positive	0.167 µg/cm ²	observed	(279)
Deodorant matrix 69 % positive	0.53 µg/cm ²	observed	(279)
Deodorant matrix 77 % positive	1.67 µg/cm ²	observed	(279)
PATCH TEST			
ED50% (in petrolatum)	32 µg/cm ²	estimated	(100)
No effect (in ethanol)	<0.0005% (0.15 µg/cm ²)	observed	(264)
No effect (in petrolatum)	<0.4 µg/cm ²	observed	(100)

Summary of results for specific fragrance ingredients

Chloroatranol (constituent of *Evernia prunastri*)

In ROAT a dose of 0.025 µg/cm² to 0.125 µg/cm² in ethanol elicited reactions in 92% to 100% of sensitised subjects.

In patch testing the ED10% was 0.0004 µg/cm².

Cinnamal

In ROAT a dose of 0.26 µg/cm² gave a response in 11% when applied as deodorant in the axilla and 82% responded to 2.63 µg/cm².

The ED50 in patch testing was 96 µg/cm².

HICC

In ROAT a dose of 0.0357 µg/cm² gave no response, while the dose that elicited a reaction in 10% of the sensitised test group (in ethanol) ranged from 0.064 µg/cm² to 1.2 µg/cm². The dose in a cream base was 4.9 µg/cm².

In ROAT a dose of 15.3 µg/cm² to 126.2 µg/cm² in ethanol elicited reactions in 61% to 89% of sensitised subjects.

The ED10 in patch testing ranged from 0.66-0.9 µg/cm².

Hydroxycitronellal

In ROAT a dose of 0.94 µg/cm² gave a response in 57% when applied in a deodorant in the axilla and 100% responded to 9.40 µg/cm².

The no-effect level in patch testing was below 0.036 µg/cm².

Isoeugenol

In ROAT a dose of 2.2 µg/cm² a response in 42% and 9.0 µg/cm² in 67%, when applied in ethanol on the arm. With a deodorant applied to the skin of the axillary, a dose of 0.167 µg/cm² caused a response in 23% and 77% reacted to 1.67 µg/cm².

The ED50 in patch testing was 32 µg/cm².

The no-effect in patch testing was below 0.15 µg/cm².

Elicitation levels have been studied for cinnamal, isoeugenol and hydroxycitronellal which are established contact allergens in humans and which already have given rise to a significant number of cases (> 100, see chapter 7). Further HICC has been studied extensively, but is considered in a separate section (chapter 11.3) of this opinion. It is however not possible to derive a safe threshold directly from the data of cinnamal, isoeugenol and hydroxycitronellal. The main reasons are that many of the test subjects reacted to all the tested doses in ROAT, which is a simulation of every day exposures. Thus it was not possible to determine the dose only eliciting responses in a few, e.g. 10% of the subjects and that only a limited number of exposure scenarios were studied.

The studies have covered few product types: hydro-alcoholic products, e.g. perfumes and deodorant roll-on matrix. The vehicle is one of many factors which influence the thresholds of allergic reactions. Also the presence of irritants and other allergens can influence the elicitation level. This means that the currently available studies do not cover all the relevant exposure scenarios. However, taking into account that dose-response investigations in sensitised patients are very complex to perform, it is not likely that much more data will become available in the near future. It is therefore necessary to exploit the full pool of elicitation data, also covering chemicals other than fragrance ingredients, to derive a more general threshold which could be used when no or insufficient data exist to set a specific threshold for a substance of concern.

General thresholds

The methodology of the different experiments has varied to some extent as different anatomical sites of exposure have been employed, different vehicles, exposure periods and cut-off points. The reason is that the studies have been performed to investigate various clinical and scientific aspects of allergic contact reactions and not for formal regulatory requirements. Some studies are small and for this reason the precision of the estimates of thresholds is limited. In spite of this, the results of the various experiments are reasonably uniform, except for chloroatranol which had very low threshold reactions, and show that low concentrations may elicit allergic reactions.

The reasonably uniform data generated on the above fragrance ingredients are in agreement with a recent "meta-analysis" of dose-response data of different allergens, incorporating some of the same studies as mentioned above, but also other allergens, such as preservatives and metals. The ED₁₀ at patch testing varied by a factor of 7 from the lowest to the highest value and the median was 0.82 µg/cm² if the three outliers formaldehyde (1997), nickel (1999) and methyldibromo glutaronitrile (2004) were left out and 0.84 µg/cm² if included (see Table 11-6 and Figure 11-2 below: (280)). An explanation of these results could be that thresholds in elicitation is less dependent on the antigenic properties of the individual substance (inherent potency) than thresholds of induction and more on the level of sensitivity of the individual, i.e. the level of T-cell clones able to recognise the antigen, which is not present in naïve not-sensitised, individuals. This seems plausible, based on both the recent clinical evidence (280) and guinea pig QSAR evidence (281). It provides the basis for a general approach in establishing safe thresholds for substances of concern.

The consequences of a limit of 0.8 µg/cm² for the product types most important for fragrance allergy are calculated below.

The calculation is based on:

- The generally safe exposure level, which is the median ED₁₀ value (the dose which will elicit allergic contact dermatitis in 10% of sensitised eczema patients) under patch test conditions: 0.8 µg/cm² (280).

- Exposure doses and exposure areas from SCCS notes of guidance 7th revision (282) [Tables 2 and 3] and Technical dossier Quantitative Risk Assessment from RIFM (274).

Equation:

Safe concentration in product = (Generally safe exposure level (0.8 µg/cm²)/daily exposure to product (µg/cm²/day)) x 100 (for %).

Table 11-5: Concentration limits in different product types based on 0.8 µg/cm² allergen as a 'generally safe exposure level', if specific dose-response data are unavailable.

	Estimated daily exposure level (g) (Table 3 SCCS NoG)	Mean exposed skin surface (cm²) (Table 2 SCCS NoG)	Exposure /cm²/day in grams	Exposure /cm²/day in µg (1g= 1x10⁶ µg)	Concentration limit in product % in product: (GEL/daily exposure) x 100
Body lotion	7.82 g	15,670 cm ²	0.000499	499	0.16%
Face cream	1.54 g	565 cm ²	0.002725	2725	0.03%
Hand cream	2.16 g	860	0.002511	2511	0.03%
Deodorant aerosol spray ethanol based	1.43 g	200 cm ²	0.007150	7150	0.01%
Perfume spray	not given	?	0.00221 ¹⁾	2210	0.04%

Note: 1) 2.21 mg/cm²/day from Technical dossier Quantitative Risk Assessment.

The estimated daily use of the various product categories in Table 11-5 are based on the SCCS Notes of Guidance (see above), except for perfume, for which no value is given. This value is taken from the Technical Dossier on Quantitative Risk Assessment from RIFM.

Generally the estimated use of different products is higher in the IFRA/RIFM assessments than in SCCS Notes of Guidance.

Table 11-6: Overview of dose-response studies and thresholds for eight allergens, after (280).

ED₁₀ patch test values from each of the 16 selected studies with 95 % confidence intervals with the allergens chromium (283), MCI/MI (Kathon[™] CG) (284), nickel (285), methyl dibromoglutaronitrile (MDBGN) (286), hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (102, 224, 263), isoeugenol (264, 279) and formaldehyde (287). The shaded values were considered as outliers.

Study	Number of patients	ED₁₀ (µg/cm²)	95 % interval
MCI/MI	12	1.05	0.17–2.27
Formaldehyde	20	20.1	4.09–43.9
Nickel 1997	24	1.58	0.32–4.04
Nickel 1998	19	0.8	0.078–2.59

Study	Number of patients	ED ₁₀ (µg/cm ²)	95 % interval
Nickel 1999	26	7.49	2.42–14.5
Nickel 2005	13	0.74	0.066–2.38
Nickel 2007	20	0.82	0.13–2.37
Cobalt 2005	11	0.44	0.033–1.3
Chromium	17	1.04	0.0033–5.55
Isoeugenol 2001	24	1.48	0.22–4.74
Isoeugenol 2005	13	0.23	0.0073–1.32
HICC 2003	18	0.85	0.062–3.26
HICC 2007	14	1.17	0.043–5.05
HICC 2009	17	0.66	0.052–2.35
MDBGN 2004	19	0.025	0.00021–0.19
MDBGN 2008	18	0.50	0.052–1.69

Note: The ED₁₀ value is the concentration which elicits an allergic reaction in 10% of a group of sensitised individuals under patch test conditions.

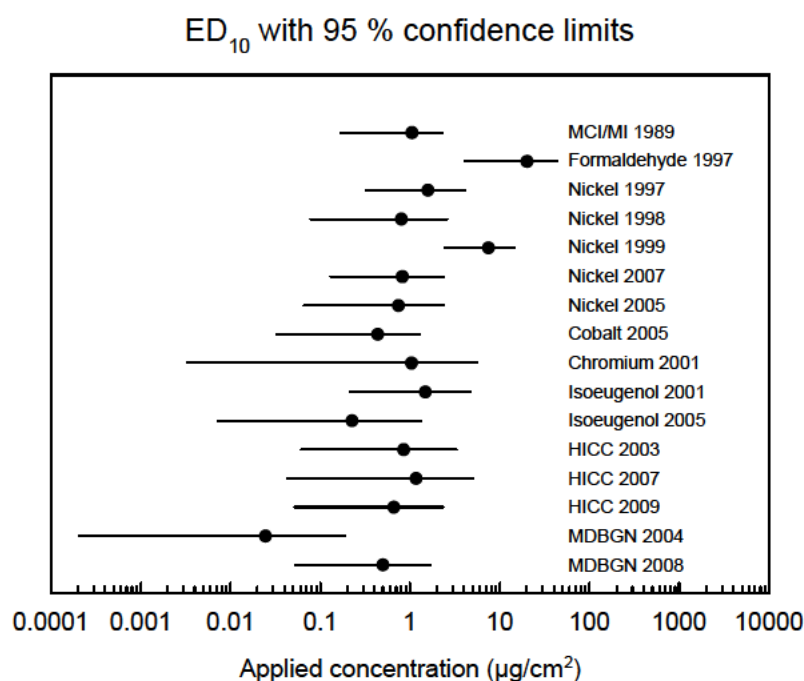


Figure 11-2: The threshold data with 95% confidence intervals from Table 11-6 presented graphically, after (280).

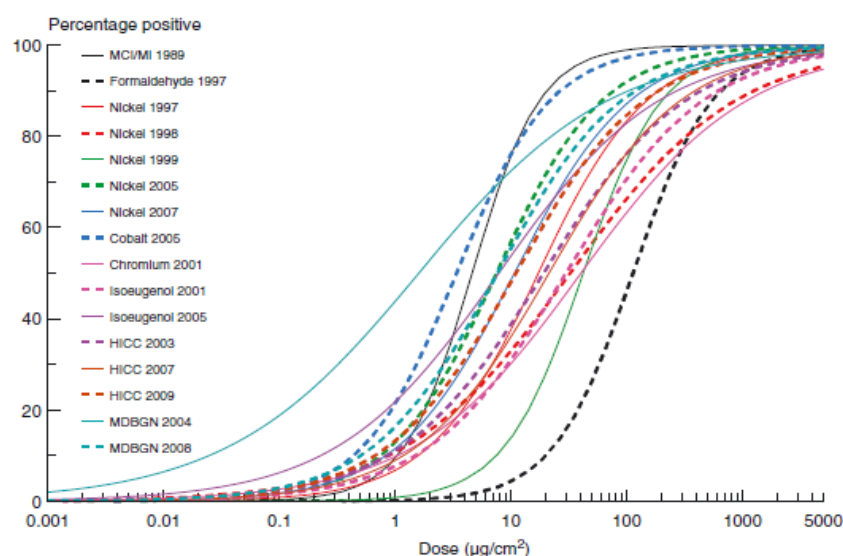


Figure 11-3: The fitted dose-response curves from the studies in Table 11-6, which are the basis for estimation of the ED10 value, after (280).

The meta-analysis above has shown that the median elicitation dose by patch testing for 10% of sensitised individuals was $0.8 \mu\text{g}/\text{cm}^2$. In the model data for the fragrance substances isoeugenol and HICC was included. The two studies on isoeugenol and the three studies on HICC gave an average ED10 value of $0.85 \mu\text{g}/\text{cm}^2$ and $0.89 \mu\text{g}/\text{cm}^2$ with a range 0.23-1.48. This means that even if the model was used for these substances individually the result would be very similar to the general threshold value.

The data from cinnamal and hydroxycitronellal studies was not incorporated in the model because: (i) serial dilution patch testing was done in petrolatum for cinnamal, making the dosing less exact; (ii) and only seven patients participated in the hydroxycitronellal study, while a criteria for inclusion in the model was ten participants (280).

According to the above calculations, a limit of $0.8 \mu\text{g}/\text{cm}^2$ for the product types of most importance for fragrance allergy corresponds to concentrations of 100 to 400 ppm (0.01-0.04%) for deodorants, perfume spray, hand and face lotions. For body lotion the general threshold was 0.16%. However, it does not seem meaningful in the context of contact allergy to distinguish between different types of creams, as a body cream would be applied with the hands and the relevant parameter in contact allergy is dose per area skin and not total dose.

A general threshold would have to take into consideration the uncertainties in quantification of exposure and safe thresholds as well as the possibilities of aggregate exposures and exposure to chemically similar substances. Therefore in setting one general threshold the product category carrying the highest risk of sensitisation and elicitation, which is deodorants, was chosen to drive the generation of the threshold. This means that a threshold of $0.8 \mu\text{g}/\text{cm}^2$ is equal to 0.01% or 100 ppm (see Table Table 11-1 and the related text), the lowest of the threshold values derived.

The approach taken by the SCCS is based on scientific evidence published in peer-reviewed journals (283)(284)(285)(286)(102, 224, 263)(264, 279)(287) in the past 20 years. The meta-analysis deriving the general threshold limit at $0.8 \mu\text{g}/\text{cm}^2$ limit has been published (280) in a peer-reviewed journal. The use of threshold limits based on elicitation data is a well established methodology which has been applied (with success) in EU to prevent further cases of induction and elicitation (primary and secondary prevention) in the case of nickel allergy, chromium in cement, chromium in shoes in

Germany, dimethyl fumurate in consumer items and also in part in IFRA guidelines e.g. concerning HICC.

The elicitation threshold model is based on 16 studies of 8 allergens, two of which are fragrance ingredients. It includes data from moderate to extreme allergens with a median EC3 value of 1.2.

The 11 fragrance allergens to which the limit is suggested to apply range from extreme to moderate with median EC3 value of 4.8, although in the case of coumarin an EC3 value could not be established.

Thus in general the potency profile of the fragrance substances of concern is not very different from those included in the model to provide the suggested general safe threshold.

The approach is targeting the relevant end-point, namely, allergic contact dermatitis. The mere consideration of potency of the allergen, according to the LLNA (EC3), is insufficient in identifying the size of the problems of contact allergy/allergic contact dermatitis. Additional information is needed from clinical and epidemiological studies, exposure assessment and dose-elicitation studies. For instance, the elicitation thresholds of e.g. HICC (EC3: 17.1) and isoeugenol (EC3: 0.54) are very similar (0.85 µg/cm² and 0.89 µg/cm², respectively) despite very different potencies. Both are frequent causes of contact allergy.

It should be noted that the general threshold is only suggested to be used for substances of concern if no specific data of sufficient quality exist to set an individual safe threshold. In cases where specific data of sufficient quality are available, these data should be used to set an individual safe threshold.

The general threshold is indicative of a safe level for the majority of sensitised individuals, but does not preclude that the most sensitive subset of the population may react upon exposure to the allergen. These levels are based on patch tests and take no account of anatomical sites of exposure, frequency of exposure or vehicle effects. Therefore, any limitations in exposures are not substitutes for providing information to the consumer about the presence of a substance in a product as a certain fraction of sensitised individuals will still need to avoid specific exposures.

Based on experience, limitations in exposure based on elicitation thresholds will, apart from helping the sensitised consumer, also significantly reduce the risk of induction. This is the case for nickel allergy, where the restrictions in the EU nickel directive are based on elicitation threshold, leading to a significant reduction in new cases of sensitisation in young women (288) and in a reduction in morbidity, i.e. elicitation (289). Another example is restriction of chromium VI in cement (290).

It is not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist, and the model providing the general use concentration limit (0.01%) has been based on chemicals only.

The SCCP concluded in 2004 that Chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in consumer products because they are extremely potent allergens (239). The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents.

11.3. Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported individual fragrance chemical causing allergy since the 1999 opinion on fragrance allergy. In total, reports of about 1500 cases have been published in the scientific literature (see chapter 7.1 and Annex I to this opinion), while the second most

frequently reported individual chemical was cinnamal with around 350 published cases. Only a minority of the cases seen by clinicians is published and only a (small) proportion of those with allergic contact dermatitis seeks or has the possibility to seek medical attention.

Natural extracts such as *Myroxylon pereirae* and turpentine (oil) have been more frequently reported, but while HICC is a synthetic fragrance chemical, where the only source of exposure is fragrances, the natural extracts are used in many other contexts than fragrances/cosmetics.

Of patients tested by the Danish monitoring network of dermatologists 2.4% were found to be allergic to HICC in 2005-2008 (with no decreasing trend from 2003 to 2007 (291)) (for more studies see chapter 4.3.2); in 70% of the cases the reaction was of current relevance, i.e. causing disease (69). This is in agreement with the results of a recent German study with HICC, where 48 out of 51 patients (94.1%) with a positive patch test reaction to HICC also reacted in a repeated open application test, simulating normal use conditions of cosmetics containing HICC (105). In a Danish study 69% of 14 HICC allergic individuals developed allergic contact dermatitis from use of cosmetics containing HICC in realistic amounts (102).

On the basis of the high frequency of allergy to HICC, in 2003 the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) recommended 0.02% (200 ppm) as maximum amount of HICC in cosmetic products (292). This has not been implemented and no restrictions apply in the Cosmetic Directive.

The fragrance industry, via the International Fragrance Association (IFRA), has its own safety guidelines. Up until 2003 HICC was used without any restriction; in 2003 a limit of 1.5% HICC in any kind of product was introduced. In 2008 this was changed according to the new risk assessment model (QRA) applied by the fragrance industry to different levels in 11 different product types derived from the QRA (see 11.1). Limits from 0.11% in lip products to 1.5% in hair styling products were set. In 2009 a further lowering was made of the limits by industry with the following reasoning: "The industry firmly believes and continues to support thresholds based on induction rather than elicitation. However, given the exceptional situation in Europe, the fragrance industry elected to take further restrictive action on this material" (293). An overview of the IFRA restrictions is given in the table below.

Table 11-7: Restriction for HICC independent of the QRA according to (293).

IFRA QRA Category	Product type that drives the category	Consumer exposure level 2003–2008 (%)	IFRA Standard July 2008 (%)	IFRA Standard July 2009 (%)
Category 1	Lip products	1.5	0.11	0.02
Category 2	Deodorants/ antiperspirants	1.5	0.15	0.02
Category 3	Hydroalcohols for shaved skin	1.5	0.60	0.2
Category 4	Hydroalcohols for unshaved skin	1.5	1.5	0.2
Category 5	Hand cream	1.5	1.0	0.2
Category 6	Mouthwash	1.5	1.5	Not applicable*
Category 7	Intimate wipes	1.5	0.3	0.02

Category 8	Hair styling aids	1.5	1.5	0.2
Category 9	Rinse-off hair conditioners	1.5	1.5%	0.2%
Category 10	Hard surface cleaners	1.5	1.5%	0.2%
Category 11	Incidental or non-skin contact	15	Not restricted	Not restricted

Note: HICC Hydroxyisohexyl 3-cyclohexene carboxaldehyde.

QRA Quantitative risk assessment.

* Not applicable because HICC is not approved for flavour use.

As an update since the presentation of the pre-consultation version of the opinion, surveillance data on HICC from two European countries have become available, covering the period 2002-2011 (IVDK/Germany (294)) and 2003-2011 (Danish contact dermatitis group (295)), respectively. The first analysis identified a slight decrease, which was considered "not overwhelming in absolute terms", namely, from 2.3% in 2002 to 2.1% in 2011 (crude prevalences, Figure 11-4). Thus, despite statistical significance, the decrease is too slight to be interpreted as relevant improvement. In the Danish study, some fluctuation around a mean prevalence of about 2.5% was noted, but no trend (Figure 11-5). It is reported that 74% of the positive reactions were regarded as clinically relevant.

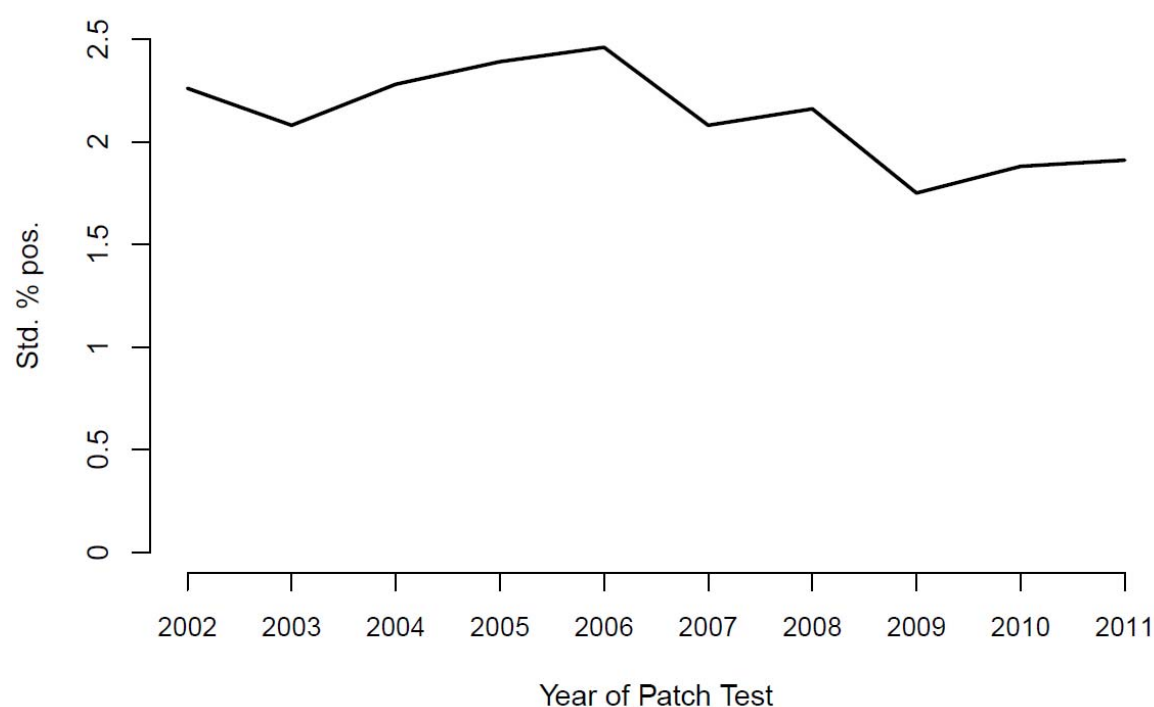


Figure 11-4: Time trend of hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitisation prevalence [standardised prevalence of positives (%)] during 2002-2011. The decrease over time is statistically significant, after **(294)**.

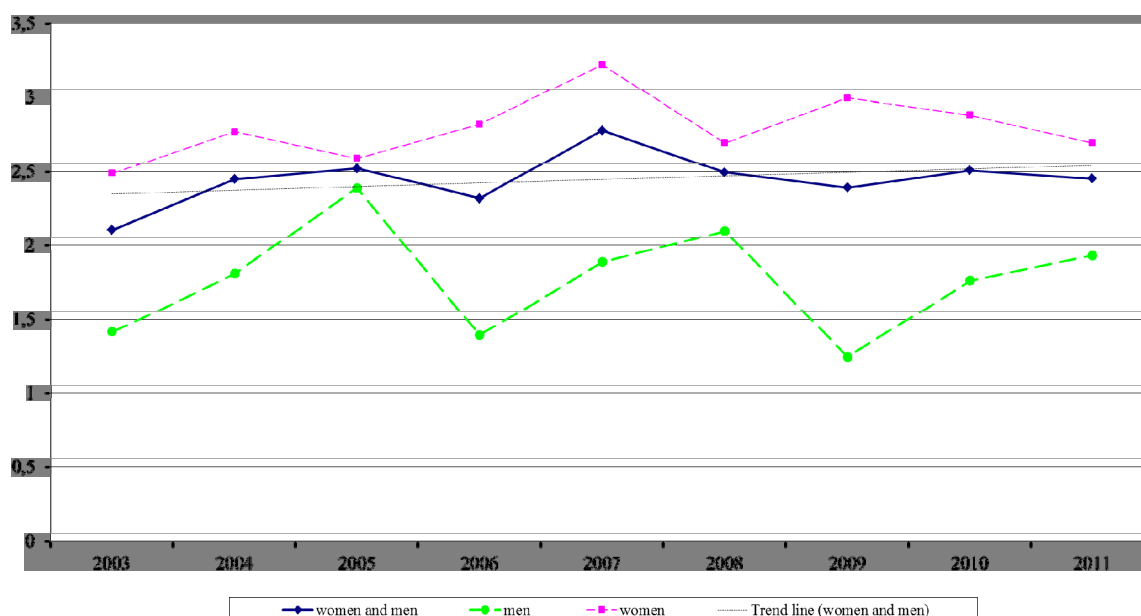


Figure 11-5: Prevalence of positive patch test reactions to hydroxyisohexyl 3-cyclohexene carboxaldehyde over time in 37 860 subjects tested by the Danish Contact Dermatitis Group (295).

11.4. Conclusion

- A dose-response relationship between exposure to contact allergens and induction of allergy (sensitisation) as well as elicitation is well established. This means that in principle, thresholds can be identified which are safe for the consumer.
- A model for dermal sensitisation quantitative risk assessment has been developed (QRA) and implemented by the fragrance industry. This model relies on thresholds, no effect or low-effect levels, established in healthy human volunteers and/or in animal experiments. The SCCP has previously reviewed this methodology and concluded that: "There is no confidence that the levels of skin sensitisers identified by the dermal sensitisation QRA are safe for the consumer."
- Elicitation data can provide thresholds indicative for the safe use of those substances which have already caused significant problems in the consumer. In this context, "safe use" means that the thresholds will protect the majority of consumers from allergic contact dermatitis, but does not preclude that the most sensitive subset of the population may react upon exposure to the allergen.
- Furthermore, based on experience from intervention studies, such thresholds will also be sufficiently low to protect (most of) the non-sensitised consumers from developing contact allergy.
- Elicitation levels have been studied specifically for the fragrance chemicals cinnamal, hydroxycitronellal and isoeugenol. These studies, however, are not adequate to derive safe thresholds for the individual substances directly from the data.
- In the absence of adequate substance specific data it is possible to use a general threshold. Based on a statistical analysis of the available data in the scientific literature, a threshold of $0.8 \mu\text{g}/\text{cm}^2$ was derived. This corresponds to 0.01% (100 ppm) limit in cosmetic products indicative for safe use.

- It is not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist and the model providing the general threshold (0.01%) has been based on individual chemicals only. However the maximum use concentration applies to the identified chemicals both if added as chemicals or as an identified constituent of a natural ingredient. This will also reduce the risk of sensitisation and elicitation from natural extracts.
- For substances for which there are no clinical data of concern, models such as the dermal sensitisation QRA approach may, after refinement and validation, be used to suggest a safe level of exposure prior to incorporation into products. However, aggregated exposures must be incorporated in the dermal sensitisation QRA model.
- HICC has for more than 10 years been recognized as an important allergen with more cases documented in the scientific literature than for any other fragrance chemical in this period. HICC has been shown to be a significant cause of disease as many of those with contact allergy to HICC had also reactions to cosmetics, which contained or were likely to contain HICC. Since 2003 attempts have been made by the fragrance industry to contain the outbreak of HICC allergy, but with no convincing success so far. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized.
- The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in consumer products because they are extremely potent allergens. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to the allergenic constituents, despite efforts to reduce the allergen content (296).

12. Data gaps and research needed

In the course of working on this opinion, the following points are highlighted as important data gaps, ordered by research area:

12.1. Clinical and epidemiological research

- Clinical data on more fragrance substances are needed to assess more fully the epidemiology of fragrance contact allergy and pin-point the culprit substances for induction and elicitation of contact allergy in man.
- Data from a broader range of EU countries on the clinical and epidemiological picture of fragrance contact allergy is needed, as difference in exposure and use habits are expected across Europe.
- A co-ordinated strategy for data collection should be developed.
- Very little is known about susceptible groups of the population, e.g. up to 10% of the European population carry mutations, which impair the skin barrier and which seem to increase the risk of fragrance allergy. Data are needed to qualify and quantify the increase in risk of susceptible groups in order to provide a better protection of all consumers.
- Aberrant enzyme activity in certain individuals, often related to genetic enzyme polymorphisms, may give an increased or reduced risk of sensitisation to prohaptens (that need enzymatic activation) in certain individuals or populations. More research into the role of relevant traits is needed.
- Dose-response data from clinical studies are available for only a few allergens. To establish individual safe levels such data are required for all established allergens of concern and covering an appropriate range of product types. This would also consolidate the basis of the use of a general threshold for safe use of fragrance allergens.
- Data on human exposure to fragrances from the use of different product categories is very scarce and therefore does not provide an optimal basis of risk assessment, e.g. exposure data on use for perfume/eau de cologne are lacking.
- Most experimental studies are done on individual fragrance ingredients, while exposure to allergens in cosmetic products is usually to mixtures of allergens. The risk of sensitisation and elicitation may depend on the mixture of substances, but very few studies on this exist. It is necessary to improve the knowledge base on cocktail effects on sensitisation/elicitation to improve the basis of risk assessment and management.
- Screening in dermatitis patients should be performed with air exposed samples of such fragrance substances that in experimental studies have been demonstrated to act as prehapten, i.e. autoxidise and form oxidation mixtures containing allergenic oxidation products.
- Patch testing should if possible, be performed with the isolated true haptens formed from prehapten and prohaptens to increase the possibility to diagnose allergy from these type of substances.
- There is a need for more experimental research to further establish the impact of the behaviour of fragrance substances when applied on the skin (including factors such as volatility, autooxidation, skin penetration, reactivity in skin and bioactivation).

12.2. Non-human studies

- Several studies in the industry submission (164) were of insufficient quality, not following the OECD guidelines.
- In some cases it was found that either very few concentrations points had been used in LLNAs, or concentrations were insufficient for achieving a 3-fold increase of the SI.

A sufficient number of doses (concentrations) should be applied in LLNAs (at least 5) so that interpolation (for deriving an EC3 value) can rely on more than two or three actual data points to be more reliable. SCCS therefore suggests a change in the OECD guideline 429. (It is important to remember that the production of unreliable data is a waste of animals.) Moreover, the maximum concentration should be high enough to achieve a > 3-fold increase in SI, as far as this is possible with the substance/vehicle combination chosen.

- Data on experimental results are often not published, but available only on file in the companies having performed the tests. Access to such results would be important for the scientific community, e.g. in the context of REACH, or independently, either to the public domain, or to a Public Trustee.
- The OECD guideline 429 recommends several vehicles. It is well known that a difference in the EC3 value can be obtained for the same substance depending on which vehicle is used in the LLNA. Thus, as an additional control, supplementary to the guideline based LLNA control, a clinically relevant solvent or the commercial formulation in which the test substance is marketed may be used.
- As long as no validated *in vitro* method exists, more research is needed. Until one or more method(s) have been decided to fulfil the requirements for substituting *in vivo* testing, the *in vivo* testing for prediction of skin sensitisation has to be used.
- Applying only mechanism-based QSAR (QMM) as a tool in non-animal based risk assessment for skin sensitisation is of limited value for fragrance substances. This is due to major information gaps in the present model when addressing substances that act via abiotic or metabolic activation, and the high incidence of such substances in fragrances. Therefore, further experimental and clinical research in the area of abiotic and/or metabolic activation of fragrance substances is needed to increase the safety for the consumer, i.e. experimental studies which include air oxidation and bioactivation.
- Further experimental investigations of the sensitisation potential of fragrance substances are needed to determine the impact of the volatility of the substance as well as the effect of the vehicle on skin penetration/absorption and reactivity.
- From a clinical perspective it is important for the individual who is sensitised to one fragrance substance to know if they must also avoid other fragrance substances that can cause allergic contact dermatitis due to cross-reactivity with the original sensitiser. Prediction of risks for cross-reactivity requires sound application of theoretical principles in combination with well-designed experimental studies. This is a field that has not been studied very much so far and needs to be focused on much more in the future.
- Quantitative structure activity relationship (QSAR) models should be further developed, combining, as appropriate, information from *in silico*, *in chemico* and *in vitro* methods as possible. Prediction of different activation pathways should be included.
- Effect estimates such as proportions of sensitised humans or animals, or mean stimulation indices, EC3 values and other derivations should ideally be accompanied by an interval estimate (confidence interval) to address precision (297).

13. Opinion

Contact allergy to fragrances is a common, significant and relevant problem in Europe. The studies since the SCCNFP opinion on fragrance allergy in consumers in 1999 (SCCNFP/0017/98) (SCCNFP 1999) have confirmed that the 26 fragrance allergens, identified by the SCCNFP, are still relevant fragrance allergens for consumers because of their exposure from cosmetic products. Additional exposure to many of these 26 fragrance allergens also occurs from the use of other consumer products, such as detergents, toys, etc. Some of these fragrance substances are also used as preservatives.

The overall trend of fragrance contact allergy appears to have been stable for the last 10 years, as some causes of fragrance allergy have decreased and others increased. From the few population-based studies, it can be estimated that the frequency of contact allergy to fragrance ingredients in the general population in Europe is 1-3%. This is based on the limited testing with eight common fragrance allergens (FM I) out of the approximately 2500 fragrance ingredients listed in CosIng and indicative of the substances that may be present in fragrance compounds. However, the real prevalence of contact allergy to fragrance substances may be higher if the testing were to be performed with the full spectrum of fragrance allergens, including oxidised substances, where relevant.

Among eczema patients in the European population, around 16% are sensitised to fragrance ingredients. The disease can be severe and generalised, with a significant impairment of quality of life and potential consequences for fitness for work.

Contact sensitisation, and its clinical manifestation, allergic contact dermatitis, can be prevented if the exposure to known contact allergens is reduced or abolished (primary prevention). Experiences so far, have indicated that not all substances that later turned out to be significant contact allergens after human exposure, were predicted by experimental studies, e.g. the preservative methyldibromo glutaronitrile and the fragrance chemical HICC. Thus, a significant exposure of the population may occur before a substance is established as an important contact allergen in man.

Elicitation of allergic contact dermatitis occurs when a consumer sensitised to a certain substance is re-exposed to the substance in question. Prevention at this stage, termed secondary prevention, can be achieved if use of the allergen in products is eliminated or reduced to a tolerable level (general prevention), or if the patients succeed in avoiding all sources of exposure (individual prevention). Ingredient listing of individual fragrance allergens has been shown to be an important tool to enable consumers with an identified allergy to reduce/avoid relevant exposures. Moreover, ingredient listing is also of great importance to ensure that an adequate diagnosis of fragrance contact allergy can be made without undue delay. If the information given on the presence of fragrance allergens is incomplete, diagnosis of fragrance contact allergy may be missed.

The SCCNFP, in its 1999 opinion, identified 26 fragrance allergens for which information should be provided to consumers concerning their presence in cosmetic products. This was implemented in the European Cosmetics legislation (298) as ingredient labelling of these 26 fragrance substances (Annex III, entries 67-92). However, safe use concentrations for these substances in cosmetic products have not yet been determined and much new evidence concerning fragrance allergy has been published since 1999. The present opinion updates the SCCNFP opinion with a systematic and critical review of the scientific literature up to October 2010. This review addresses the issue of contact allergy to fragrance substances, including natural extracts and updates the list of fragrance allergens relevant to consumers. Clinical, epidemiological and experimental studies were evaluated, as well as modelling studies performed, to establish lists of: (i) established fragrance allergens; (ii) likely fragrance allergens; and (iii) possible fragrance allergens. The review also includes fragrances, which on modification by oxidation or by enzyme mediated processes, can produce allergens. Available dose-response data have been

examined to answer whether safe thresholds can be established for the most frequent fragrance allergens.

13.1. Question 1

Does the SCCS still consider that the fragrance allergens currently listed in Annex III, entries 67-92, for labelling purposes represent those fragrance ingredients that the consumer needs to be made aware of when present in cosmetic products?

In order to answer this question, the SCCS has used clinical and epidemiological data to identify known fragrance allergens. These were categorised as *established contact allergens in humans* (see Table 13-1).

Where sufficient animal evidence was present, these substances were categorised as established contact allergens in animals (Table 13-2). For a number of other fragrance substances, combinations of limited clinical data together with SAR considerations have been applied to indicate likely fragrance allergens in man (Table 13-3). Finally, SAR has also been applied to substances that lack human data to identify fragrance chemicals that have the structural potential to be contact allergens. Substances with insufficient human data were also considered as possible fragrance allergens. For these further tests (experimental/clinical data) are required (Table 13-4).

Table 13-1: Established contact allergens in humans.

For categorisation of importance (+ to +++) see chapter 7.1. Allergens of special concern are substances where between 100 and 1,000 cases (+++) and more than 1,000 (+++++) have been published. These are set in bold. Fragrance substances identified as allergens in the 1999 opinion of SCCNFP (1) are marked with an asterisk.

"ox." = oxidised; "non-ox." = non-oxidised; "r.t." = rarely tested (see chapter 7)

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
Individual chemicals		
ACETYLCEDRENE	32388-55-9	+
AMYL CINNAMAL*	122-40-7	++
AMYL CINNAMYL ALCOHOL*	101-85-9	++
AMYL SALICYLATE	2050-08-0	+
trans-ANETHOLE	4180-23-8	+ (r.t.)
ANISE ALCOHOL*	105-13-5	+
BENZALDEHYDE	100-52-7	+
BENZYL ALCOHOL*	100-51-6	++
BENZYL BENZOATE*	120-51-4	++
BENZYL CINNAMATE*	103-41-3	++
BENZYL SALICYLATE*	118-58-1	++
BUTYLPHENYL METHYLPROPIONAL *	80-54-6	++
CAMPHOR	76-22-2 / 464-49-3	+ (r.t.)
beta-CARYOPHYLLENE (ox.)	87-44-5	Non-ox.: +, ox.: +

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
CARVONE	99-49-0 / 6485-40-1 / 2244-16-8	+ (r.t.)
CINNAMAL*	104-55-2	+++
CINNAMYL ALCOHOL*	104-54-1	+++
CITRAL*	5392-40-5	+++
CITRONELLOL*	106-22-9 / 1117-61-9 / 7540-51-4	++
COUMARIN*	91-64-5	+++
(DAMASCENONE) ROSE KETONE-4	23696-85-7	+ (r.t.)
alpha-DAMASCONE (TMCHB)	43052-87-5 / 23726-94-5	++
cis-beta-DAMASCONE	23726-92-3	+
delta-DAMASCONE	57378-68-4	+
DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	151-05-3	+
EUGENOL*	97-53-0	+++
FARNESOL*	4602-84-0	++ - +++
GERANIOL*	106-24-1	+++
HEXADECANOLACTONE	109-29-5	+ (r.t.)
HEXAMETHYLINDANOPYRAN	1222-05-5	++
HEXYL CINNAMAL*	101-86-0	++
HYDROXYISOHEXYL CARBOXALDEHYDE (HICC)* 3-CYCLOHEXENE	31906-04-4 / 51414-25-6	++++
HYDROXYCITRONELLAL*	107-75-5	+++
ISOEUGENOL*	97-54-1	+++
alpha-ISOMETHYL IONONE*	127-51-5	++
(DL)-LIMONENE*	138-86-3	++ (non-ox.); +++ (ox.)
LINALOOL*	78-70-6	++ (non-ox.) +++ (ox.)
LINALYL ACETATE	115-95-7	+ (non-ox.) ++ (ox.)
MENTHOL	1490-04-6 / 89-78-1 / 2216-51-5	++
6-METHYL COUMARIN	92-48-8	++
METHYL 2-OCTYNOATE*	111-12-6	++
METHYL SALICYLATE	119-36-8	+
3-METHYL-5-(2,2,3-TRIMETHYL-3-	67801-20-1	++ (r.t.)

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
CYCLOPENTENYL)PENT-4-EN-2-OL		
alpha-PINENE and beta-PINENE	80-56-8 and 127-91-3, resp.	++
PROPYLIDENE PHTHALIDE	17369-59-4	+ (r.t.)
SALICYLALDEHYDE	90-02-8	++
alpha-SANTALOL and beta-SANTALOL	115-71-9 and 77-42-9, resp.	++
SCLAREOL	515-03-7	+
TERPINEOL (mixture of isomers)	8000-41-7	+
alpha-TERPINEOL	10482-56-1 / 98-55-5	
Terpinolene	586-62-9	+
TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	+
TRIMETHYL-BENZENEPROPANOL (Majantol)	103694-68-4	++
VANILLIN	121-33-5	++
Natural extracts		
CANANGA ODORATA and Ylang-ylang oil	83863-30-3; 8006-81-3	+++
CEDRUS ATLANTICA BARK OIL	92201-55-3; 8000-27-9	++
CINNAMOMUM CASSIA LEAF OIL CINNAMOMUM ZEYLANICUM BARK OIL	8007-80-5 84649-98-9	++ (r.t.)
CITRUS AURANTIUM AMARA FLOWER / PEEL OIL	8016-38-4; 72968-50-4	++
CITRUS BERGAMIA PEEL OIL EXPRESSED	89957-91-5	+ (r.t.)
CITRUS LIMONUM PEEL OIL EXPRESSED	84929-31-7	++
CITRUS SINENSIS (syn.: AURANTIUM DULCIS) PEEL OIL EXPRESSED	97766-30-8; 8028-48-6	++
CYMBOPOGON CITRATUS / SCHOENANTHUS OILS	89998-14-1; 8007-02-1; 89998-16-3	++
EUCALYPTUS SPP. LEAF OIL	92502-70-0; 8000-48-4	++
EUGENIA CARYOPHYLLUS LEAF / FLOWER OIL	8000-34-8	+++
EVERNIA FURFURACEA EXTRACT*	90028-67-4	+++
EVERNIA PRUNASTRI EXTRACT*	90028-68-5	+++
JASMINUM GRANDIFLORUM / OFFICINALE	84776-64-7; 90045-94-6; 8022-96-6	+++
JUNIPERUS VIRGINIANA	8000-27-9;	++

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text
	85085-41-2	
<i>LAURUS NOBILIS</i>	8002-41-3; 8007-48-5; 84603-73-6	++
<i>LAVANDULA HYBRIDA</i>	91722-69-9	+ (r.t.)
<i>LAVANDULA OFFICINALIS</i>	84776-65-8	++
<i>MENTHA PIPERITA</i>	8006-90-4; 84082-70-2	++
<i>MENTHA SPICATA</i>	84696-51-5	++
MYROXYLON PEREIRAE	8007-00-9;	++++
<i>NARCISSUS SPP.</i>	diverse	++
<i>PELARGONIUM GRAVEOLENS</i>	90082-51-2; 8000-46-2	++
<i>PINUS MUGO/PUMILA</i>	90082-72-7 / 97676-05-6	++
<i>POGOSTEMON CABLIN</i>	8014-09-3; 84238-39-1	++
<i>ROSE FLOWER OIL (ROSA SPP.)</i>	Diverse	++
SANTALUM ALBUM	84787-70-2; 8006-87-9	+++
TURPENTINE (oil)	8006-64-2; 9005-90-7; 8052-14-0	++++
VERBENA ABSOLUTE	8024-12-2	++

Table 13-2: Fragrance substances categorised as established contact allergens in animals.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)
Individual chemicals			
Allyl phenoxyacetate	7493-74-5	none	3.1
p-tert. -Butyldihydrocinnamaldehyde	18127-01-0	none	4.3
CYCLAMEN ALDEHYDE	103-95-7	none	22
Dibenzyl ether	103-50-4	none	6.3
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	116-26-7	limited	7.5
trans-2-Hexenal	6728-26-3	none	2.6
2-Hexylidene cyclopentanone	17373-89-6	none	2.4
HEXYL SALICYLATE	6259-76-3	negative	0.18
p-Isobutyl- α -methyl hydrocinnamaldehyde	6658-48-6	none	9.5

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)
Isocyclocitral	1335-66-6	none	7.3
α -Methyl cinnamic aldehyde	101-39-3	none	4.5
METHYLENEDIOXYPHENYL METHYLPROPANAL	1205-17-0	none	16.4
METHYLUNDECANAL	110-41-8	none	10
2-Methoxy-4-methylphenol	93-51-6	none	5.8
4-Methoxy- α -methyl benzenpropanal	5462-06-6	none	23.6
METHYL OCTINE CARBONATE	111-80-8	limited	2.5
Perillaldehyde p-Mentha-1,8-dien-7-al	2111-75-3	none	8.1
PHENYLACETALDEHYDE	122-78-1	limited	3
Natural extracts			
Jasminum Sambac Flower CERA / Extract / Water	91770-14-8	none	35.4

Table 13-3: Fragrance substances categorised as likely contact allergens by combination of evidence.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
AMBRETTOLIDE	7779-50-2	limited	none	+
CARVACROL	499-75-2	limited	none	+
Citrus paradisi §	8016-20-4	none	R43	n.a.
CUMINALDEHYDE	122-03-2	limited	none	+
CYCLOPENTADECANONE	502-72-7	limited	none	+
trans-trans-delta-DAMASCONE	71048-82-3	limited	none	+
2,4-dimethyl-3-cyclohexen-1-carboxaldehyde §	68039-49-6	none	R43	+
DIMETHYLTETRAHYDRO BENZALDEHYDE	68737-61-1	limited	none	+
ETHYL VANILLIN	121-32-4	limited	none	+
HELIOTROPINE	120-57-0	limited	none	+
ISOAMYL SALICYLATE	87-20-7	limited	none	++
ISOLONGIFOLENEKETONE	33407-62-4	limited	none	+
Longifolene §	475-20-7	none	R43	+
Mentha arvensis §	68917-18-0	none	R43	n.a.
METHOXYCITRONELLAL	3613-30-7	limited	none	+
METHYL CINNAMATE	103-26-4	limited	none	++
METHYLIONANTHEME	55599-63-8	limited	none	+

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
5-METHYL-alpha-IONONE	79-69-6	limited	none	+
MYRCENE	123-35-3	limited	none	++
MYRTENOL	515-00-4	limited	none	+
NEROL	106-25-2	limited	none	++
Nerolidol (isomer not specified)	7212-44-4	limited	none	++
NOPYL ACETATE	128-51-8	limited	none	+
PHYTOL	150-86-7	limited	none	+
RHODINOL	6812-78-8	limited	none	+
trans-ROSE KETONE-5	39872-57-6	limited	none	++

§ Substances/natural mixtures were classified as R43, according to the submission by IFRA. The evidence on which this classification was based was not available to the SCCS, so the validity of classification cannot be assessed. Nevertheless, the four substances/substance mixtures should be treated as *likely contact allergens*.

n.a.: not applicable (natural mixture)

Table 13-4: Fragrance substances categorised as possible contact allergens.

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
Individual chemicals				
CYCLOHEXYL ACETATE	622-45-7	limited	none	0
ETHYLENE DODECANEDIOATE	54982-83-1	limited	none	0
HYDROXYCITRONELLOL	107-74-4	limited	none	0
METHOXYTRIMETHYLHEPTANOL	41890-92-0	limited	none	0
METHYL p-ANISATE	121-98-2	limited	none	0
METHYL DIHYDROJASMONATE	24851-98-7	limited	none	0
PHENETHYL ALCOHOL	60-12-8	limited	none	0
PHENYLPROPANOL	122-97-4	limited	none	0
AMYL CYCLOPENTANONE	4819-67-4	negative	none	+
BENZYL ACETATE	140-11-4	negative	none	+
6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	93939-86-7	negative	none	+
3a,4,5,6,7,7a-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	54830-99-8	negative	none	+
alpha-IONONE	127-41-3	negative	none	+
beta-IONONE	79-77-6	negative	none	+
METHYL IONONE (mixture of	1335-46-2	negative	none	+

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INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC value (min; %)	SAR
isomers)				
TERPINEOL ACETATE (Isomer mixture)	8007-35-0	negative	none	+
alpha-TERPINYL ACETATE	80-26-2	negative	none	+
CITRONELLYL NITRILE	51566-62-2	none	none	++
alpha-CYCLOHEXYLIDENE BENZENEACETONITRILE	10461-98-0	none	none	+
DECANAL	112-31-2	none	none	++
DIHYDROMYRCENOL	18479-58-8	none	none	+
3,7-DIMETHYL-1,6-NONADIEN-3-OL	10339-55-6	none	none	++
2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	28219-61-6	none	none	+
GERANYL ACETATE	105-87-3	none	none	++
HEXAHYDRO-METHANOINDENYL PROPIONATE	68912-13-0	none	none	+
IONONE isomeric mixture	8013-90-9	none	none	+
ISOBERGAMATE	68683-20-5	none	none	+
METHYL DECENOL	81782-77-6	none	none	+
TRICYCLODECENYL PROPIONATE	17511-60-3	none	none	+
OXACYCLOHEXADECENONE	34902-57-3	none	none	++
VERDYL ACETATE	2500-83-6/ 5413-60-5	none	none	+
trans-beta-Damascone	23726-91-2	none	none	+
gamma-Damascone	35087-49-1	none	none	+
Citronellal	106-23-0	none	none	++
Phenethyl salicylate	87-22-9	none	none	++
Natural extracts				
ACORUS CALAMUS ROOT OIL	84775-39-3	Limited	none	
CEDRUS DEODARA WOOD OIL	91771-47-0	Limited	none	
CITRUS AURANTIUM AMARA LEAF OIL	72968-50-4	Limited	none	
CITRUS TANGERINA ...	223748-44-5	Limited	none	
CYMBOPOGON NARDUS / WINTERIANUS HERB OIL	89998-15-2; 91771-61-8	Limited	none	
ILLICIUM VERUM FRUIT OIL	84650-59-9	Limited	none	
LAVANDULA SPICA	97722-12-8	Limited	none	

INCI name (or, if none exists, perfuming name according to CosIng)	CAS number	Human evidence: see text	EC 3 value (min; %)	SAR
LITSEA CUBEBA	90063-59-5	Limited	none	
PELARGONIUM ROSEUM	90082-55-6	Limited	none	
SALVIA spp.	Diverse	Limited	none	
TAGETES PATULA	91722-29-1	Limited	none	
THYMUS spp.	84929-51-1	Limited	none	
VETIVERIA ZIZANOIDES	8016-96-4; 84238-29-9	Limited	none	

Regarding the above categorisation of fragrance substances, the following aspects need to be considered when interpreting an outcome other than established contact allergen in humans:

- If human evidence is negative, there is still a potential sensitisation risk, as in this set of substances the number of (consecutive) patients tested was low, i.e. up to a few hundred.
- If EC3 values are given as higher (>) than a certain value (see 8.3), an exact EC3 could not be established, as the substance had been tested in too low concentration(s). In these cases, the substances have not been categorised as 'established contact allergen in animals'.
- For SAR, the categories of prediction are: non-sensitiser (0); possible-sensitiser (+); predicted sensitiser (++); and not predictable (n.p.). (For details see Table 9-3 and Table 9-4). SAR predictions are only considered when human and animal data are limited or missing.
- Several substances are currently banned from the use in cosmetic products by Annex II of the Cosmetics Directive, based on concerns regarding one or more toxicological endpoints. While available clinical evidence regarding this set of substances is listed in Annex I to this opinion, these substances have not further been evaluated.

Fragrance ingredients listed in Table 13-1 clearly have caused disease in man, and based on the clinical experience alone, these 82 substances were classified as established contact allergens in humans, 54 individual chemicals and 28 natural extracts (mixtures of chemicals), including all 26 fragrance allergens identified by SCCNFP in 1999. For a number of other substances, no patch test data were available, but positive animal data, obtained by a validated guideline method (LLNA) addressing hazard, indicate that a – yet not quantified – risk for humans is very likely to exist, given sufficient exposure. In other cases only in a relatively small number of patients has been tested positively ('limited human evidence'). Here, combination with SAR analyses corroborates the conclusion that these substances, too, are sufficiently qualified to be regarded as 'likely fragrance allergens'.

Of those 82 substances identified as established contact allergens in humans, 12 chemicals (listed in Table 13-5) and eight natural extracts are considered of special concern as they have given rise to at least 100 reported cases. These substances pose a particularly high risk of sensitisation to the consumer and are further considered in the answer of question 2. One substance, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), was shown to be the cause of allergic contact dermatitis in more than 1500 reported cases since 1999. The number of cases is only those reported in scientific publications, and therefore the actual number of cases is severely under-estimated.

Table 13-5: Established fragrance contact allergens of special concern (single chemicals only).

Cinnamal
Cinnamyl Alcohol*
Citral
Coumarin
Eugenol*
Farnesol*
Geraniol*
Hydroxycitronellal
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)
Isoeugenol*
Limonene (oxidised)
Linalool* (oxidised)

*including their respective esters

The established contact allergens in animals (Table 13-2) and the likely contact allergens, identified based on a combination of limited evidence from man together with positive SAR predictions (Table 13-3), are predicted to cause disease in man given sufficient exposure.

Information on the presence of all the substances given in Table 13-1, Table 13-2 and Table 13-3 in cosmetic products is important in order to enable aimed testing of patients with contact dermatitis and to diagnose fragrance allergy without delay. Further, this information is important to the sensitised consumer as it will enable them to avoid cosmetic products, which they may not tolerate.

Substances given in Table 13-4 are possible contact allergens and further data are required to judge if these are contact allergens in humans and give rise to contact allergy in consumers.

Conclusions - Question 1

The studies since the SCCNFP Opinion on fragrance allergy in consumers (1) have confirmed that the fragrance allergens currently listed in Annex III, entries 67-92 are still relevant fragrance allergens for the consumers from their exposure to cosmetic products.

The review of the clinical and experimental data shows that many more fragrance substances than those identified in the SCCNFP opinion of 1999 have been shown to be sensitisers in humans. A comprehensive list of established contact allergens in humans is given in Table 13-1.

Moreover, animal experiments indicate that additional fragrance substances can be expected to be contact allergens in humans, although human evidence is currently lacking.

Additionally, limited human and/or animal evidence together with structure activity relationship analysis suggests that other fragrance ingredients may be a cause of concern with regard to their potential of causing contact allergy in humans.

Ingredient listing is important in clinical practice for the management of patients who are allergic to one or more of the listed fragrance chemicals. It is also important for the

patients in order to avoid future exposure to fragrance contact allergens which they may not tolerate.

The SCCS considers that those substances itemised in Table 13-1, Table 13-2 and Table 13-3 represent those fragrance ingredients that the consumer should be made aware of when present in cosmetic products.

Substances known to be transformed (e.g. hydrolysis of esters) to known contact allergens should be treated as equivalent to these known contact allergens. The combined concentration of the alcohol and its ester must be considered regarding exposure. Important indicative, but not exhaustive, examples include isoeugenol and its esters, geraniol and its esters, eugenol and its esters, and linalool and its esters.

13.2. Question 2

Can the SCCS establish any threshold for their safe use based on the available scientific data?

Dose-response relationships exist between exposure to contact allergens and the proportion of consumers who will become sensitised to an allergen (i.e. induction), as well as the proportion who will suffer from allergic contact dermatitis (elicitation). For a number of recognised contact allergens in man, dose-elicitation studies on sensitised individuals are available. These studies indicate that it is in principle possible to derive exposure levels that the majority of sensitised individuals will tolerate. The SCCS considers that thresholds based on elicitation levels in sensitised individuals will be sufficiently low to protect both the majority of sensitised individuals as well as most of the non-sensitised consumers from developing contact allergy and limit the risk of induction.

Among the established chemical fragrance allergens, 12 were identified as posing a high risk of sensitisation to the consumer (Table 13-5), i.e. more than 100 reported cases. For these substances, limitation of exposure would help to protect sensitised consumers from developing allergic contact dermatitis.

In cases where specific data of sufficient quality on threshold levels for a particular allergen are available, these data should be used to set an individual safe threshold. However, when such quality data are not available and a substance has been identified to pose a high risk of sensitisation to the consumer, a general threshold limit can be applied.

Dose-response studies have been performed with only four of these fragrance substances (HICC, isoeugenol, cinnamal and hydroxycitronellal). In addition, such a study has also been performed on chloroatranol, a potent allergen in *Evernia prunastri* and *Evernia furfuracea*. These studies, however, are not adequate to derive safe thresholds for the individual substances directly from the data.

If no adequate data are available, for substances posing a high risk to the consumer (like the 12 listed in Table 13-5), the use of a general threshold may be considered. A threshold of 0.8 µg/cm² has been derived based on a statistical analysis of the available data in the scientific literature, including two fragrance allergens. This corresponds to 0.01% (100 ppm) limit in cosmetic products indicative for safe use. This approximation may hold for weak to strong allergens. However, some strong and extreme sensitisers may require lower individual thresholds. As an example, chloroatranol, present in the natural product *Evernia prunastri* and in *Evernia furfuracea*, has been shown to have an elicitation threshold of 0.0004 µg/cm² under experimental conditions similar to those yielding above results. On the other hand, for very weak sensitisers, this generic threshold may be too conservative.

The model providing the general threshold of 100 ppm has been based on single substances only and no general safe level for the natural extracts of concern can be

identified, but the maximum use concentration applies to the identified fragrance allergens also when present in the natural extract.

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) has been the most frequently reported chemical causing fragrance allergy since the 1999 opinion on fragrance allergy. In total, reports of more than 1500 cases have been published in the scientific literature (see chapter 7.1 and Annex I), which will severely underestimate the actual prevalence in the population. HICC has been shown to be a significant cause of disease as many of those with contact allergy to HICC had also reactions to cosmetics, which contained or were likely to contain HICC. The SCCP concluded in 2003 that 200 ppm of HICC would be tolerated by the majority of sensitised individuals and this level of exposure would have a low potential to induce sensitisation (241). Since 2003 attempts have been made by the fragrance industry to contain the outbreak of HICC allergy, but with no convincing success so far. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Chloroatranol and atranol are the main allergenic components of *Evernia prunastri* and *Evernia furfuracea*. The SCCS concluded in 2004 (239) that these should not be present in cosmetic products, due to their exceptionally high sensitisation potential. Attempts to effectively reduce the content of these compounds in "oak moss abs." (300) have largely failed to reduce contact allergy to *Evernia prunastri* and *Evernia furfuracea* and the data presented in this opinion show that the number of cases remains high.

Conclusions - Question 2

There are two components to the safety of fragrance ingredients in terms of contact allergy. First, the need to eliminate or reduce induction of contact allergy (primary prevention), which, when it occurs, is life long. Secondly, the need to eliminate or reduce elicitation reactions (secondary prevention) on the skin of those individuals who are already sensitised. Human dose elicitation experiments have hitherto been performed only for a very small number of substances. It is unlikely that more of these studies will be performed due to experimental and subject recruitment difficulties.

For individual substances, no levels that could be considered safe for the majority of consumers could be established from the available data.

The dose elicitation studies available indicate that a general level of exposure of up to 0.8 µg/cm² (0.01%) may be tolerated by most consumers with contact allergy to fragrance allergens. The SCCS considers that this level of exposure could be efficient in limiting elicitation unless there is substance specific data, either experimental or clinical, to the contrary.

Such a threshold based on elicitation levels in sensitised individuals will be sufficiently low to protect both sensitised individuals as well as most of the non-sensitised consumers from developing contact allergy.

The SCCS is of the opinion that for substances identified as posing a high risk to the consumer and for which no individual thresholds could be derived (Table 13-5), the general threshold of 0.01% would limit the problem of fragrance allergy in the consumer significantly.

It was not possible to provide a safe threshold for natural extracts of concern, as no specific investigations exist and the model providing the general threshold (0.01%) has been based on individual chemicals only. However the SCCS considers that the maximum use concentration applies to the above identified fragrance allergens also when present in the natural extract. This will also reduce the risk of sensitisation and elicitation from natural extracts.

It is important to stress that this general threshold, although limiting the problem, does not preclude that the most sensitive segment of the population may react upon exposure to these levels. Hence, this threshold does not remove the necessity for providing information to the consumer concerning the presence of the fragrance substance in cosmetics.

In the case of hydroxyisohexyl 3-cyclohexene carboxaldehyde, in 2003 the SCCP suggested that levels of up to 200 ppm would be tolerated by the majority of sensitised individuals. Recent voluntary restrictions (recommendations to lower use concentrations, at least for some product types, to the level recommended by the SCCS in 2003) are not reflected in available evidence and are considered insufficient. The SCCS considers that the number of cases of HICC allergy documented over the last decade is exceptionally high and that continued exposure to HICC by the consumer is not considered safe, even at concentrations as low as 200 ppm. Therefore, HICC should not be used in consumer products in order to prevent further cases of contact allergy to HICC and to limit the consequences to those who already have become sensitized. The SCCP concluded in 2004 that chloroatranol and atranol, the main allergenic constituents of *Evernia prunastri* and *Evernia furfuracea*, should not be present in products for the consumer. The persistently high frequency of contact allergy to *Evernia prunastri* and *Evernia furfuracea* noted in eczema patients does point to a persisting problem with exposure to allergenic constituents, despite efforts to reduce the allergen content (296). The SCCS is of the opinion that the presence of the two constituents, chloroatranol and atranol, in cosmetic products are not safe.

13.3. Question 3

Can the SCCS identify substances where processes (e.g. metabolism, oxidation and hydrolysis) may lead to cross-reactivity and new allergens which are relevant for the protection of the consumer?

Many fragrance substances can act as prehaptenes or prohaptens, forming allergens which are more potent than the parent substance by abiotic and/or metabolic activation, and thus increasing the risk of sensitisation.

Experimental and clinical studies have shown that there are fragrance substances that act as prehaptenes, i.e. their sensitisation potency is markedly increased by air exposure due to oxidation (autooxidation). Non/low-sensitising compounds are thereby transformed into more potent sensitisers. Limonene, linalool, linalyl acetate, alpha-terpinene and geraniol have all been identified as prehaptenes. These fragrance substances are common in scented cosmetics as well as in household products. The clinical studies show that the exposure to allergens formed due to autooxidation causes significant contact allergy in consumers. Patch testing with oxidised limonene and oxidised linalool shows that these substances rank among the most common contact allergens.

In the SAR analyses performed in this work by the SCCS, fragrance compounds with structural alerts that indicate that they are possible prehaptenes have been identified (Table 9-1, Table 9-2). In such cases further thorough investigations are needed. It is also important to investigate the stability of the primary oxidation products (the hydroperoxides) formed from various structures of fragrance compounds. The stability of these compounds can have great impact on the sensitisation potency of the oxidised compound as they are strong sensitisers. However, the secondary oxidation products (aldehydes and epoxides) can also be important sensitisers depending on the overall structure of the compound as was demonstrated for oxidised geraniol.

Air oxidation of prehaptenes can be prevented to a certain extent by measures during handling and storage of the ingredients and final products to avoid air exposure, and/or by addition of suitable antioxidants. The autooxidation rate depends not only on the compound itself, but also on its purity. The prevention of autooxidation using antioxidants

needs thorough investigation because antioxidants can exert their function by being oxidised instead of the compound that they protect and might thereby be activated to skin sensitising derivatives after oxidation. As antioxidants are now frequently used at elevated concentrations in scented products due to a growing awareness of the problem of autoxidation, there is a risk that sensitisation caused by the antioxidants will rise. One of the most used antioxidants is butylated hydroxytoluene (BHT) which is considered a minimal risk for sensitisation in the concentrations used but nevertheless, with increased concentrations and usage, the risk of sensitisation could increase.

It should be noted that, to decrease the risk for sensitisation in the population, the possibility to reduce the sensitisation potency by preventing autoxidation is important also for a direct acting hapten or prohaptens, if a further activation by air oxidation to more allergenic compounds has been shown.

Based on the clinical data, oxidised limonene and oxidised linalool are allergens of high concern (Table 13-5) which pose a high risk of sensitisation to the consumer. For these substances the presence of the oxidised fraction represented by the peroxide content should not be higher than 10 ppm. Alternatively, the suggested general threshold dose/area of 0.8 µg/cm² (100 ppm in cosmetic products) could be applicable to the total oxidised fraction, i.e. not only peroxides but also secondary oxidation products such as aldehydes and epoxides.

Compounds that are bioactivated by metabolising enzymes to haptens are referred to as prohaptens. Established prohaptens of clinical importance are cinnamyl alcohol, geranial, geraniol, eugenol, isoeugenol and alpha-terpinene.

Table 13-6: Known prehaptens and prohaptens.

Fragrance substance	Activation by air oxidation	Bioactivation (oxidation)	Bioactivation (hydrolysis)
Cinnamyl alcohol		x	
Eugenol		x	
Eugenyl acetate		x	x
Geranial	x	x	
Geraniol	x	x	
Geranyl acetate	x	x	x
Isoeugenol		x	
Isoeugenyl acetate		x	x
Limonene	x		
Linalool	x		
Linalyl acetate	x		
alpha-Terpinene.	x	x	

When bioactivation occurs, the risk of cross-reactivity should be considered. An increased complexity in the cross-reactivity pattern is obtained when a compound could act both as a prehaptens and a prohaptens. For instance, it is known that cinnamyl alcohol and cinnamal can cross-react due to the formation of common sensitising substances. The same applies to geraniol and citral.

In case derivatives of a fragrance substance are used, it must be taken into account that the derivative could be transformed into the parent or a cross-reacting compound. For such derivatives the same rules as for the corresponding parents should apply, unless the

stability of the derivative has been demonstrated. In particular, hydrolysis of esters to the corresponding alcohols can cause cross-reactions. Acetate esters of eugenol, isoeugenol and geraniol are frequently used in cosmetics.

To be able to predict the sensitisation potency of prohaptens, steps of bioactivation have to be included in the predictive tests.

Activation of individual compounds to various haptens increases the risks of cross-reactivity between chemicals and also causes difficulties in prediction of these risks. Prediction of risks requires sound application of theoretical principles in combination with well designed experimental studies. Based on the acquired knowledge, qualified suggestions using structure activity relationship (SAR) regarding many fragrance substances have been made (Table 9-1 to Table 9-3). However, as the stability of formed oxidation products (mainly hydroperoxides) is important for the sensitisation potency, the SAR hypotheses must be followed by experimental investigations for the actual compounds.

Conclusions - Question 3

Many fragrance substances can act as prehaptens or prohaptens, forming allergens which are more potent than the parent substance by abiotic and/or metabolic activation. Activation can thus increase the risk of sensitisation. Fragrances with published data showing the formation of sensitising compounds by autooxidation, bioactivation or both include the following (see also Table 13-6).

Fragrance substances of clinical importance known to be prehaptens and to form sensitising compounds by air oxidation are limonene, linalool, and linalyl acetate.

Fragrance substances of clinical importance known to be prohaptens and to form sensitising compounds by metabolic transformation are cinnamyl alcohol, eugenol, isoeugenol and isoeugenyl acetate.

Fragrance substances of clinical importance with published data known to be both prehaptens and prohaptens and to form sensitising compounds by air oxidation (prehaptens) and by metabolic transformation are geraniol and alpha -terpinene.

A fragrance substance that sensitises without activation but forms more potent sensitising compounds by air oxidation and also by metabolic transformation is geranial (one isomer of citral).

In the case of prehaptens, it is possible to prevent activation outside the body to a certain extent by different measures, e.g. prevention of air exposure during handling and storage of the ingredients and the final product and by the addition of suitable antioxidants. When antioxidants are used, care should be taken that they will not be activated themselves and thereby form new sensitisers.

The possibility to reduce the sensitisation potency by preventing air oxidation is important also for a direct acting hapten or prohaptens, if a further activation by air oxidation to more allergenic compounds has been shown.

In the case of prohaptens, the possibility to become activated is inherent to the molecule and activation cannot be avoided by extrinsic measures. Activation processes increase the risk for cross-reactivity between fragrance substances. Cross-reactivity has been shown for certain alcohols and their corresponding aldehydes, i.e. between geraniol and geranial (citral) and between cinnamyl alcohol and cinnamal.

Cross-reactivity is also expected between ester derivatives and their parent alcohols, as the esters will be hydrolysed by esterases in the skin. Esters of important contact allergens that can be activated by hydrolysis in the skin are isoeugenyl acetate, eugenyl acetate and geranyl acetate which all are known to be used as fragrance ingredients.

The substances presented above are based on current knowledge and should be seen as indicative and illustrative of the general problem. As substances with structural alerts for acting as pro- and/or prehapten are quite common among the fragrance substances listed (see Tables 9-1 and 9-2), the possibility for activation to generate new potent allergens should be considered.

The SCCS is of the opinion that substances known to be transformed (e.g. by oxidation either via air oxidation or via bioactivation) to known contact allergens should be treated as equivalent to these contact allergens, i.e the same restrictions and other regulatory requirements should apply, unless specific data exist that allow for an individual assessment. Important indicative examples include limonene, linalool, linalyl acetate, geraniol, geranial, alpha-terpinene, eugenol, isoeugenol and cinnamyl alcohol.

14. List of abbreviations

ACD	Allergic contact dermatitis
alc.	Alcohol (as vehicle)
CI	Confidence interval
CLP	Classification, labelling and packaging
coloph.	Colophonium
DCs	Dendritic cells
EC	European Commission
ESSCA	European Surveillance System on Contact Allergies
EDT	Eau de toilette
EDP	Eau de perfume
EU	European Union
FM	Fragrance mix
GC	Gas chromatography
GPMT	Guinea pig maximisation test
HICC	Hydroxyisohexyl 3-cyclohexene carboxaldehyde
HRIPT	Human repeat insult patch test
IFRA	International Fragrance Association (www.ifraorg.org)
IVDK	Information Network of Departments of Dermatology (www.ivdk.gwdg.de)
INCI	International Nomenclature on Cosmetic Ingredients
LCs	Langerhans cells
LLNA	Local lymph node assay
MPR	<i>Myroxylon pereirae</i> resin
NACDG	North American Contact Dermatitis Group
OECD	Organization of Economic Co-operation and Development
ox.	oxidised
pet.	Petrolatum (as vehicle)
ppm	parts per million (10000 ppm = 1%)
PPV	Positive predictive value
PR	Prevalence ratio
PT(ed)(ing)	Patch test(ed) (ing)
QMM	Quantitative mechanistic model
QRA	Quantitative risk assessment
(Q)SAR	(Quantitative) structure activity relationship
REACH	Registration, Evaluation, Authorisation and restriction of CHemicals
RIFM	Research Institute for Fragrance Materials (www.rifm.org/)

ROAT	Repeated open application test
SC	Single constituents (of one of the fragrance mixes)
SCCS	Scientific Committee on Consumer Safety
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products
SCCP	Scientific Committee on Consumer Products
UK	United Kingdom
US(A)	United States (of America)
UV	Ultraviolet

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Annex I - Catalogue of fragrance allergens**Contents**

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Single chemicals

Often, results with the single constituents of the FM I or, yet more rarely, FM II, are presented in one paper. As the main ordering of this annex is by allergen, core information on these studies is presented in a tabular format and referenced by a unique acronym in the single sections, to avoid redundancy. Regarding nomenclature, terms which are often not officially an INCI Name but Perfuming Name as listed by CosIng are used. "Current Regulation" refers to the EU Cosmetics Directive only.

Table 55: Background information on studies reporting results with (all) single constituents of the FM I (**amyl cinnamal, cinnamyl alcohol, cinnamal, eugenol, geraniol, hydroxycitronellal, isoeugenol, EVERNIA PRUNASTRI**)

Reference	Country	Study period, Patients	Comments by reviewers
Larsen 2002 c (1)	7 industrial countries worldwide	Prior to 2002 n=218 patients with known contact allergy to fragrance ingredients	Test concentrations identified as non-irritating in serial dilution testing in 20 healthy volunteers
Utrecht 1999 (2)	Utrecht, The Netherlands	1994-1998 n=757 patients with suspected ACD to cosmetics	All patients tested with FM I and single constituents
Sheffield 1999 (3)	UK	1994-1995 n=744, 40 of these positive to FM I and tested with single constituents	
IVDK 2007 (4)	Germany + one centre in Austria and Switzerland each	01/2003 – 12/2004, n=1658 to 21325, see text, consecutive patients	
Hungary 2002 (5)	Hungary, multicentre study,	1998-1999, n=3604 patients	recruitment not clear, presumably consecutive patients
Groningen 2009 (6)	Groningen, The Netherlands	04/2005-06/2007 n=320	patients selected according to history or site suspicious of contact allergy to fragrance ingredients
IVDK 2010 (7)	Germany, Switzerland and one centre in	2005-2008 n=36961 tested with FM I, n=4167 with FM II and	

	Austria	all SC	
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Table 56: Results of PTing with single constituents of the FM I in patients positive to the FM I (as percent)

N(pos) to FM I, ref.	<i>Evernia prun.</i>	Isoeu g.	Hydroxy citron.	Cinna mal	Cinnamy l alcohol	Eugen ol	Gera- niol	Alpha- amyl cinnam al
N=160 (5)	13.1%	14.8 %	2.5%	8.1%	20.6%	8.8%	7.5%	5.0%
N= 991 (8)	18.4%	11.2 %	10.1%	6.1%	6.1%	6.6%	4.6%	2.4%
N=50 (2)	19.6%	14.3 %	8.9%	8.9%	7.1%	5.4%	2.7%	0%
n=40 Sheffield 1999 (3)	30%	20%	2.5%	12.5 %	10%	5%	0%	0%
N=226 Coimbra 2000 (9)	22.1%	19.9 %	6.6%	13.3 %	7.9%	14.6 %	8.4%	4.4%
N=655 IVDK 2010 (7)	29.8%	18.0 %	12.8%	11.6 %	9.6%	6.7%	4.7%	2.8%

Table 57: Background information on studies reporting results with (all) single constituents of the FM II (**citronellol, citral, coumarin, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), Farnesol, alpha-Hexyl-cinnamic aldehyde**)

Reference	Country	Study period, Patients	Comments by reviewers
IVDK 2007 (4)	Germany + one centre in Austria and Switzerland each	01/2003 – 12/2004, n=1658 to 21325, see text, consecutive patients	
EU 2005 (10)	6 European centres	10/2002 – 06/2003, n=1701	Applied in consecutive patients
Groningen 2009 (6)	Groningen, The Netherlands	04/2005-06/2007 n=320	patients selected according to history or site suspicious of contact allergy to fragrance ingredients

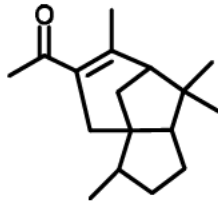
IVDK 2010b (11)	Germany, Switzerland and one centre in Austria	2005-2008 n=35633 tested with FM II, n=2217 with all SC	
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Table 58: Background information on studies reporting results with several fragrance compounds not, or only partly, corresponding to mixes (later created) or with essential oils

Reference	Country	Study period, Patients
deGroot 2000 (12)	The Netherlands (multicentre)	09/1998-04/1999 n=1825 consecutive patients
An 2005 (13)	South Korea (multicentre)	04/2002 – 06/2003 n=422 consecutive patients
Sugiura 2000 (14)	Nagoya, Japan	1990-1998 n=1483 patients with suspected cosmetic dermatitis
Frosch 1995 (15)	11 European depts.	Prior to 1995 n=1069 consecutive patients
Frosch 2002 a (16)	6 European depts.	10/1997-10/1998 n=1855 consecutive patients
Frosch 2002 b (17)	6 European depts.	Prior to 2002 n=1606 consecutive patients
Coimbra 2000 (9)	Portugal	07/1989-06/1999 n=226 with FM I SC n=67 also with other fragrances
Larsen 1977 (18)	US	1977 n=20 “perfume-sensitive patients”
Larsen 2001 (19)	worldwide multicentre	? (prior to 2001) n=178 patients with known contact allergy to fragrance ingredients
Belsito 2006 (20)	North American (5 US, 1 Canadian) depts.	2003 n=1603 patients
NACDG 2009 (21)	US and Canada	2005-2006 n= 4454 patients
Wöhrl 2001 (22)	“FAZ” clinic Vienna	1997-2000 n=747 of 2660 consecutive patients tested with special series
EECDRG 1995 (15)	European, multicentre	Different fragrances, tested in 2 concentrations, in sets of about 100 patients each in different centres
Goossens 1997 (23)	Leuven, Belgium	1978-1987 n=111 “Japanese perfume series” (highly selected patients)

Reference	Country	Study period, Patients
Malten 1984 (24)	Dutch multicentre	N=182 patients with suspected cosmetic dermatitis tested with 22 fragrance compounds
DeGroot 1985 (25)	Dutch	N=179 patients with suspected cosmetic dermatitis tested with 16 fragrance compounds
Rudzki 1976 (26)	Warsaw, Poland	N=200 consecutive patients
Rudzki 1986 (27)	Warsaw, Poland	N=86 patients of 299 (of 5315) patients with positive reaction to FM I tested with essential oils series
Santucci 1987 (28)	Rome, Italy	N=1500 consecutive patients; n=63 reacting positively to FM I re-tested with extended fragrance series
Nakayama 1974 (after (29))	Japan	N=183 patients with cosmetic dermatitis
IVDK 2010c (30)	Germany, Switzerland and one centre in Austria	15682 patients tested with at least one essential oil in different test series
Trattner/David (31)	Tel Aviv, Israel	N=641 consecutive patients

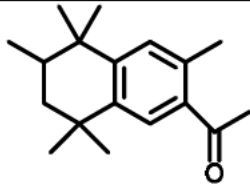
Catalogue of single chemicals evaluated

ACETYLCEDRENE	
CAS # 32388-55-9	
EC # 251-020-3	
1-[(3R,3aR,7R,8aS)-2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl]-ethanone	
Other names 1-(2,3,4,7,8,8a-Hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl)-, [3R-(3a,3aβ,7β,8aα)]-Ethanone; 1H-3a,7-Methanoazulene, Ethanone deriv.; Acetyl-α-cedrene; Lixetone; Vertofix	

Current regulation: /

Clinical data:
In the Frosch 2002 a study, a total of 0.2% had positive PT reactions (16). In the Frosch 1995 dose-finding pilot study, 1 positive reaction to 1% and none to 5% "Vertofix ®" in pet., tested in 100 consecutive patients in Stockholm, were observed (15). In a case report, a 28-year-old patient with axillary dermatitis after using 2 different deodorants tested positive not only to HICC, but also to acetyl cedrene (tested 10.8% in diisopropylene glycol (20 healthy controls negative) (32). In this case report it is stated that "Acetyl cedrene (Vertofix Coeur) is a complex reaction mixture of which a principal constituent is methyl cedryl ketone".

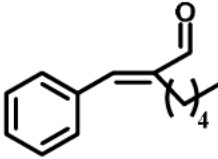
Additional information:
Acetyl cedrene (Vertofix®, IFF) is a complex mixture obtained from cedar wood oil by the acetylation of terpenes. The principal component of acetyl cedrene is methyl cedryl ketone (CAS 32388-55-9). It is a "top 100" substance (IFRA, pers. comm.2010)

6-ACETYL-1,1,2,4,4,7-HEXAMETHYLTETRALINE	
CAS # 21145-77-7	
EC # 216-133-4 / 244-240-6	
1-(5,6,7,8-Tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-ethanone	
AHMT (perfume), AHTN, Extralide, Fixolide, Musk tonalid, NSC 19550, Tentarome, Tetralide, Tonalid, Tonalide.	

Current regulation: Annex III, part 1, entry 182

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% "Tonalide ®" in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

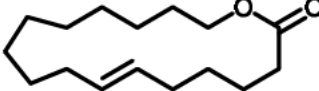
AMYL CINNAMAL	
CAS # 122-40-7	
EC # 204-541-5	
2-(Phenylmethylene)-heptanal	
Cinnamaldehyde, α-amyl- (4CI); Cinnamaldehyde, α-pentyl- (6CI,7CI,8CI); 2-(Phenylmethylene)heptanal; 2-Benzylideneheptanal; Amylcinnamaldehyde; Amylcinnamic acid aldehyde; Amylcinnamic aldehyde; Flomine; Jasminal; Jasminaldehyde; Jasmine aldehyde; NSC 6649; Pentylcinnamaldehyde; α-Amyl-β-phenylacrolein; α-Amylcinnamal; α-Amylcinnamaldehyde; α-Pentylcinnamaldehyde	

Current regulation: Annex III, part 1, entry 67

Clinical data:
In the "background information" section of the 1999 opinion (33), amyl cinnamal (synonymous: alpha amyl cinnamaldehyde) has been classified as frequently reported contact allergen because it has been identified as a cause of allergic reactions in persons with eczema from cosmetic products.

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=4, i.e., 0.2% (95% CI: 0.1 – 0.5%) positive reactions to this compound (1% pet.) in 2062 consecutively PTed patients (4). In the Groningen 2009 study, no positive reactions to this allergen, tested at 2% pet., were observed (6). The Larsen 2001 study yielded 2.3% positive reactions in 178 patients with known contact allergy to fragrance ingredients (test concentration: 5% pet.) (19). In the Wöhr 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=2 (0.3%) positive reactions to amyl cinnamal (22). The IVDK 2010 study, 0.26% (95% CI: 0 – 0.60%) of 1214 consecutively tested patients reacted to the compound, while 0.61% (95% CI: 0.36 – 0.86%) of 4375 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7).

Additional information:
It is a "top 100" substance and classified as R43 (IFRA, pers. comm. 2010).

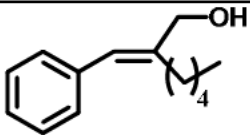
AMBRETTOLIDE	
CAS # 7779-50-2	
EC # 231-929-1	
Oxacycloheptadec-7-en-2-one	
1-Oxa-7-cycloheptadecen-2-one; 16-Hydroxy-6-hexadecenoic acid lactone; 16-Hydroxy-6-hexadecenoic acid ω-lactone	

Current regulation: /

Clinical data:
The Larsen 2001 study, using omega-6-hexadecenlactone (HDL, 5% pet.) as test concentration, diagnosed 3.4% positive reactions in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information:

Ambrettolide is 1 of 2 components of Ambrette seed oil (obtained from *Hibiscus abelmoschus* L., *Malvaceae*) responsible for the musk odour. In Surburg/Panten, the compound has the chemical name (Z)-7-hexadecen-16-olide (or Hexadec-7-en-16-olide according to CosIng), CAS 123-69-3 (34).

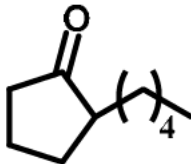
AMYL CINNAMYL ALCOHOL	
CAS # 101-85-9	
EC # 202-982-8	
2-(Phenylmethylene)-heptan-1-ol, 2-Benzylidene- (6CI,8CI)1-heptanol; 2-Amyl-3-phenyl-2-propen-1-ol; 2-Benzylidene-1-heptanol; 2-Pentyl-3-phenyl-2-propen-1-ol; Buxinol; α-Amylcinnamic alcohol; α-Amylcinnamyl alcohol	

Current regulation: Annex II, Part 1, entry 74

Clinical data:
In the "background information" section of the 1999 opinion, amyl cinnamyl alcohol is mentioned to cross-react with amyl cinnamal. Moreover, this compound has been identified as a cause of allergic reactions in a notable number of persons with eczema from the use of cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.4% (95% CI: 0.1 – 0.7%) positive reactions in 1977 consecutively PTed patients (4). The IVDK 2010 study, 0.79% (95% CI: 0.54 – 1.04%; percentages standardised for age and sex) of 5650 patients PTed reacted to the compound (7). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6).

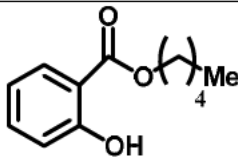
Additional information: A RIFM review is available (35) where selected clinical studies published until 1994 were considered.

AMYLCYCLOPENTANONE	
CAS # 4819-67-4	
EC # 225-392-2	
2-Pentylcyclopentanone 2-Pentyl-1-cyclopentanone; 2-Pentylcyclopentanone; 2-Pentylcyclopenten-1-one; 2-n-Amylcyclopentanone; 2-n-Pentyl cyclopentanone; Delphone	

Current regulation: /

Clinical data:
In the Larsen 2001 study, none of 178 patients with contact allergy to fragrance ingredients reacted positively to this ingredient, PTed at 5% pet. (19).

Additional information: /


AMYL SALICYLATE	
CAS # 2050-08-0	
EC # 218-080-2	
Pentyl-2-hydroxybenzoate	
Amyl ester salicylic acid, (4CI); Pentyl ester salicylic acid, (6CI,8CI); 2-Hydroxybenzoic acid pentyl ester; Amyl salicylate; NSC 403668; NSC 44877; NSC 46125; Pentyl salicylate	

Current regulation: /

Clinical data:

In the Frosch 2002 a study, a total of n=3 (0.2%) had positive PT reactions (16). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% amyl salicylate and 1 positive reaction to 5% amyl salicylate were observed in 100 consecutive patients patch tested in Stockholm (15).

Additional information:
A RIFM review is available (36). It is a "top 100" substance (IFRA, pers. comm.2010)

trans-ANETHOLE	
CAS # 4180-23-8	
EC # 224-052-0 / 203-205-5	
1-Methoxy-4-(1E)-1-propen-1-yl-benzene	
(E)-p-Propenyl-anisole (8CI); (E)-1-Methoxy-4-(1-propenyl)-benzene; 1-Methoxy-4-(1E)-1-propenyl-benzene (9CI); (E)-1-(4-Methoxyphenyl)propene; (E)-1-p-Methoxyphenylpropene; (E)-Anethol; (E)-Anethole (REACH, EINECS); E-Anethole (INCI); 1-Methoxy-4-[(1E)-1-propenyl]benzene; (E)-1-Methoxy-4-(1-propenyl)-benzene (CosIng); NSC 209529; trans-1-(4-Methoxyphenyl)-1-propene; trans-1-(p-Methoxyphenyl)-1-propene; trans-1-(p-Methoxyphenyl)propene; trans-1-p-Anisylpropene; trans-4-(1-Propenyl)anisole; trans-Anethol; trans-Anethole; trans-p-Anethole; trans-p-Methoxy-β-methylstyrene	

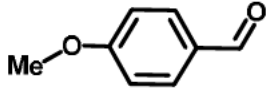
Current regulation: /

Clinical data:

A case of a 64 year old patient, who developed severe cheilitis and a loss of taste has been described (37). Both were reversible after the cessation of use of previous toothpastes. The patch test was strongly positive to anethole (isoform not given) 5% pet.; this was found an ingredient of the causative toothpaste. Two cases of occupational allergic contact dermatitis occurring in a traditional cake factory due to anise oil have been described, both testing (strongly) positive to anise oil (5% o.o.) and anethole (5% pet.) (38).

Additional information:
It is a "top 100" substance (IFRA, pers. comm.2010). trans-Anethole can be purified from star anise oil (34, 39), see 3.2., and is the main component of anise, star anise

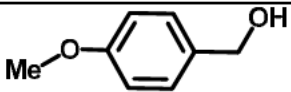
and fennel oils (38)

ANISALDEHYDE	
CAS # 123-11-5	
EC # 204-602-6	
4-Methoxy-benzaldehyde	
p-Methoxybenzaldehyde; p-Anisaldehyde; 4-Anisaldehyde; Aubepine; Crategine; NSC 5590; Obepin; p-Anisic aldehyde; Anisic aldehyde; p-Formylanisole.	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

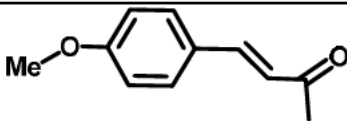
ANISYL ALCOHOL	
CAS # 105-13-5	
EC # 203-273-6	
4-Methoxy-benzenemethanol	
p-Methoxy-benzyl alcohol (8CI); (4-Methoxyphenyl)methyl alcohol; 4-(Hydroxymethyl)anisole; 4-(Methoxyphenyl)methanol; 4-Methoxy- α -hydroxytoluene; 4-Methoxybenzenemethanol; 4-Methoxybenzyl alcohol; Anise alcohol; Anisic alcohol; NSC 2151; [4-(Methoxy)phenyl]methanol; p-(Methoxyphenyl)methanol; p-Anisalcohol; p-Anisyl alcohol; p-Methoxybenzyl alcohol	

Current regulation: Annex III, part 1, n° 80

Clinical data:
In the "background information" section of the 1999 opinion, anisyl alcohol is classified as "less frequently reported allergen"; 2 studies were identified where 3 and 4 cases, respectively, with cosmetic dermatitis due to contact allergy to anisyl alcohol had been reported (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded $n=1$, i.e., 0.1% (95% CI: 0.00 – 0.3%) positive reactions in 2004 consecutively PTed patients, patch test concentration: 1% pet. (4). Similar results were obtained in the following period, with $n=1$ (and $n=3$ irritant and $n=6$ doubtful) reactions in 986 patients tested with 1% in pet. (30). In the Groningen 2009 study, no positive reactions to this allergen, tested at 5% pet., were observed in 320 patients (6). This test concentration has been regarded as relatively high by Hostynek and Maibach (40). The test concentration of Anisyl Alcohol has been further validated by Bruze et al. and 10% in pet was recommended as a non-irritant concentration for routine investigations (40a).

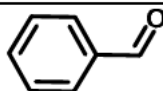
Additional information: /

ANISYLIDENE ACETONE	
CAS # 943-88-4	
EC # 213-404-9	
4-(4-Methoxyphenyl)-3-Buten-2-one	
1-(p-Methoxyphenyl)-1-buten-3-one; 4-(4-Methoxyphenyl)-3-buten-2-one; 4-(p-Methoxyphenyl)-3-buten-2-one; 4-Methoxybenzalacetone; 4-Methoxybenzylideneacetone; 4-Methoxystyryl methyl ketone; 4'-Methoxybenzylideneacetone; Anisalacetone; Methyl p-methoxystyryl ketone; NSC 31752; NSC 7946; p-Anisalacetone; p-Methoxybenzalacetone; p-Methoxybenzylideneacetone; p-Methoxystyryl methyl ketone	

Current regulation: Annex III, part 1, n° 443

Clinical data:
In the Malten 1984 study, 1.1% of 182 patients displayed a positive PT reaction to anisylidene acetone 2% pet. (24)

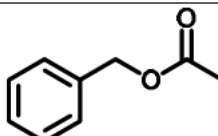
Additional information: /

BENZALDEHYDE	
CAS # 100-52-7	
EC # 202-860-4	
Benzaldehyde	
Artificial Almond Oil; Benzaldehyde FFC; Benzenecarbonal; Benzenecarboxaldehyde; Benzoic acid aldehyde; Benzoic aldehyde; NSC 7917; Phenylformaldehyde; Phenylmethanal	

Current regulation: /

Clinical data:
 In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions to benzaldehyde 5% pet. (22). The IVDK 2010 study, 6 weak positive reactions were observed, i.e., 0.16% (95% CI: 0.03 – 0.29%; percentages standardised for age and sex) of 2820 patients PTed reacted to the compound (7). A review is available in the Int. J. Toxicol. (41). In the case of a 19 year old pastry maker, Seite-Bellezza et al. report on immediate reactions to MP, cinnamal and benzaldehyde (tested at 5% pet.) subsiding after a few hours, in line with the patient's history (42).

Additional information: /

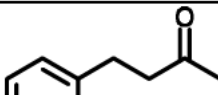
BENZYL ACETATE	
CAS #140-11-4	
EC # 205-399-7 / 202-940-9	
Benzyl acetate	
Benzyl ester acetic acid; Benzyl alcohol, acetate (6CI); (Acetoxymethyl)benzene; Benzyl ethanoate; NSC 4550; Phenylmethyl acetate; Methyl Phenylacetate; α-Acetoxytoluene ; Methyl alpha-Toluate	

Current regulation: /

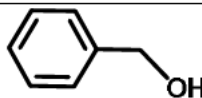
Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% benzyl acetate in pet., tested in 100 consecutive patients in Odense, DK, were observed (15). Benzyl acetate is a component of several natural mixtures, for example a major constituent of Narcissus abs., and a minor constituent of Jasmine abs. (17).

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

BENZYL ACETONE	
CAS # 2550-26-7	

EC # 219-847-4	
4-Phenyl-2-butanone	
4-Phenylbutan-2-one (REACH, EINECS); Benzylacetone; Methyl 2-phenylethyl ketone; Methyl phenethyl ketone; NSC 44829; NSC 813M; Phenethyl methyl ketone; 1-Phenyl-3-butanone; 2-Phenylethyl methyl ketone	
Current regulation: /	
Clinical data: /	
Additional information: It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (43).	

BENZYL ALCOHOL	
CAS # 100-51-6	
EC # 202-859-9	
Phenylmethanol	
Benzyl alcohol; (Hydroxymethyl)benzene; Benzenecarbinol; Benzylic alcohol; NSC 8044; Phenylcarbinol; Benzenemethanol; Phenylmethyl alcohol; Sunmorl BK 20; TB 13G; α-Hydroxytoluene; α-Toluenol	
Current regulation: Annex III, part 1, n° 45; Annex VI, part1, n ° 34	

Clinical data:
In the "background information" section of the 1999 opinion, benzyl alcohol is classified as allergen frequently causing allergic reactions. It has been found to cause allergic reactions in 1.2 to 15% of patients with eczema from cosmetic products (33). A CIR expert panel review is available in the Int. J. Toxicol. (44).

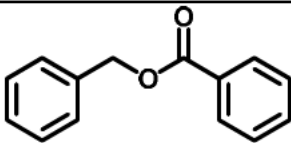
Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.7%) positive reactions in 2166 consecutively PTed patients (4). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen (6).

Both in terms of case reports (45-47) and clinical epidemiology data (0.22 % [95% CI: 0.16 – 0.28%] positive tested with benzyl alcohol in the context of a "topical drugs" series, n=26448 (7)) the relevance of this alternative exposure is highlighted. In a study from Alicante, Spain, 86 selected patients were tested with benzyl alcohol, yielding 2 positive reactions (48).

After application of saline soaks preserved with benzyl alcohol onto his stasis dermatitis, a 53 year old patient developed a rash, which was, according to test results obtained by J. D. Guin and J. Goodman, at least partly due to an immediate hypersensitivity to benzyl alcohol, as verified by an intense urticarial reaction at the test site lasting several days (49). According to 2 cases reported by A. A. Fisher, PT-proven, relevant delayed type hypersensitivity is not associated with immediate reactions in scratch or intradermal tests (50). D. W. Shaw describes a patient with allergic contact dermatitis caused by benzyl alcohol in a hearing aid impression material and in topical medications (51). Another contribution points to covert exposures to benzyl alcohol even in products labelled "fragrance free" (52) probably because benzyl alcohol is used as preservative, or an essential oil containing benzyl alcohol is used as cosmetic ingredient.

Additional information:

Benzyl alcohol is a component of several natural mixtures, including Myroxylon pereirae resin, which have been used for extraction, but is nowadays synthesised (53). It is permitted in certain foodstuffs (liquors: < 100 mg/l, sweets and cakes: < 250 mg/kg) under the coding "E 1519" (http://www.zusatzstoffe-online.de/zusatzstoffe/317.e1519_benzylalkohol.html, last accessed 2009-11-27). In addition to being a fragrance compound (which may be used, even in relatively high concentration, to scent topical medications (54)), benzyl alcohol is used as antioxidant in topical therapeutics or cosmetics. The German "Rote Liste" (<http://www.rote-liste.de>, last accessed 2009-11-11), for instance, lists 205 specialties containing benzyl alcohol. Benzyl alcohol may be used up to 1.0% as a preservative in cosmetic products according to the Cosmetic Directive 76/768/EEC

BENZYL BENZOATE	
CAS # 120-51-4	
EC # 204-402-9	
Benzyl benzoate	
Benzyl ester benzoic acid; Ascabin; Ascabiol; Benylate; Benzyl benzenecarboxylate; Benzyl benzoate; Benzyl phenylformate; Benzylets; Colebenz; NSC 8081; Nicca Sunsolt LM 7EX; Novoscabin; Pelemol B66; Peruscabin; Phenylmethyl benzoate; Scabagen; Scabanca; Scabcare BB; Scabide; Scabiozon; Scobenol; Vanzoate; Venzonate	

Current regulation: Annex III, part 1, n° 85

Clinical data:

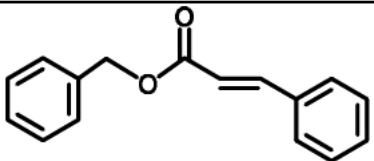
In the "background information" section of the 1999 opinion, benzyl benzoate is classified as "less frequently reported allergen"; in several studies, only single cases had been reported in each, except for patients sensitive to MP (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=1, i.e., 0.1% (95% CI: 0.00 – 0.3%) positive reactions in 2003 consecutively PTed patients, test concentration 1% pet. (4). In the subsequent period (2005-2008), n=1062 patients were tested in the IVDK 2010 study, with no positive reactions (7). In the Groningen 2009 study, no positive reactions to this allergen, tested at 5% pet., were observed in 320 patients (6). Thus, the pooled proportion of positive patch test reactions is 1 / 3385 (0.03%, exact upper 1-sided 95% CI: 0.14%)

Additional information:

Benzyl benzoate naturally occurs in MP resin and ylang-ylang oil. Nowadays it is synthesised and used for a variety of purposes (53). These include use as a scabicide (one brand specialty on the German market, using a concentration of 10% for children and 25% for adults), possibly with some differences among European countries. In France, a combination of benzyl benzoate 10% and sulfur 2% is reported to be used most often (55). Hausen et al. review the older literature and mention a study identifying 1 sensitised patient in 73 patients treated for scabies (details not given) (53). According to the mandatory factsheet (see PDF "benzylbenzoate_infosheet_DE.pdf") dermatitis after anti-scabies treatment is "rare", in a range between 1:1000 and 1:10000.

It is a "top 100" substance (IFRA, pers. comm.2010).

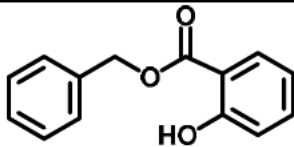
BENZYL CINNAMATE	
CAS # 103-41-3	
EC # 203-109-3	
Benzyl 3-phenylprop-2-enoate	
Benzyl ester cinnamic acid; 3-phenyl-phenylmethyl ester 2-propenoic acid; 3-Phenyl-2-propenoic acid benzyl ester; Benzyl 3-phenylpropenoate; Benzyl γ-phenylacrylate; Cinnamein; NSC 11780; NSC 44403	

Current regulation: Annex III, part 1, n° 81

Clinical data:
In the "background information" section of the 1999 opinion, benzyl cinnamate (synonymous: benzyl 3-phenyl-2-propenoate, cinnamein) is classified as "less frequently reported allergen"; one study of patients with contact allergy to cosmetic products was identified and further a study where benzyl cinnamate associated with contact sensitisation to MP (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.6%) positive reactions in 2042 consecutively PTed patients, test concentration 5% pet. (4). The IVDK 2010 study, n=4 weak positive were observed, amounting to 0.12% (95% CI: 0 – 0.25%; percentages standardised for age and sex) of 2872 patients PTed reacted to the compound (7). In the Groningen 2009 study, no positive reactions to this allergen, using the same test concentration, were observed in 320 patients (6). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions (22).

Additional information: A RIFM review is available (56).

BENZYL SALICYLATE	
CAS # 118-58-1	
EC # 204-262-9	
Benzyl 2-hydroxybenzoate	
Salicylic acid, Benzyl ester; Benzoic acid, 2-Hydroxy-, phenylmethyl ester; Benzyl o-hydroxybenzoate; NSC 6647	

Current regulation: Annex III, part 1, n° 75

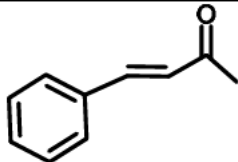
Clinical data:

In the “background information” section of the 1999 opinion (33), benzyl salicylate is classified among the frequent allergens, with 0.2 to 10% of patients with eczema from cosmetic products testing positively. In one study, benzyl salicylate accounted for 75% of reactions to commercial products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded n=2, i.e. 0.1% (95% CI: 0.01 – 0.4%) positive reactions in 2041 consecutively PTed patients (test concentration 1% pet.) (4). The IVDK 2010 study, 2 of 3775 patients PTed reacted weakly positive to the compound (7). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen, tested at 2% pet. (6). In the deGroot 2000 study, 10 of 1825 consecutive patients tested positive to benzyl salicylate (2% pet.), of these, 3 were not detected by the FM I (12). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=3 (0.4%) positive reactions (22). Trattner/David found 2 positive cases in 641 consecutive eczema patients (31). In a study from Alicante, Spain, 86 selected patients were tested with benzyl salicylate, yielding 2 positive reactions (48).

Additional information:

It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010). A RIFM review is available, including internal results on, e.g. HRIPT, and a review of LLNA results, where benzyl salicylate is classified as “weak” allergen (57).

BENZYLIDENEACETONE	
CAS # 122-57-6	
EC # 204-555-1	
4-Phenyl-3-buten-2-one	
4-Phenylbut-3-en-2-one; 2-Butenone, 4-Phenyl- (2CI); Ketone, Methyl styryl (7CI); 1-Phenyl-1-buten-3-one; 2-Phenylethenyl methyl ketone; 2-Phenylvinyl methyl ketone; 4-Phenyl-3-buten-2-one; 4-Phenyl-3-butene-2-one; 4-Phenylbutenone; Acetocinnamone; Benzalacetone; Benzylideneacetone; Methyl 2-phenylvinyl ketone; Methyl phenylvinyl ketone; Methyl styryl ketone; Methyl β-styryl ketone; NSC 5605; Styryl methyl ketone	

Current regulation: Annex II, n° 356

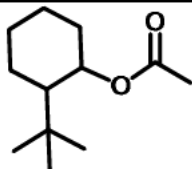
Clinical

data:

In the Malten 1984 study, none of 182 patients displayed a positive PT reaction to

benzylidene acetone 0.5% pet. (24).

Additional information: /

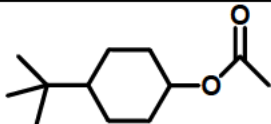
2-TERT-BUTYLCYCLOHEXYL ACETATE	
CAS # 88-41-5	
EC # 201-828-7	
2-(1,1-dimethylethyl)cyclohexyl acetate	
Cyclohexanol, 2-(1,1-dimethylethyl)-, acetate ; Cyclohexanol, 2-Tert-butyl-, acetate; 2-Tert-Butylcyclohexanol acetate; Verdox; o-Tert-Butylcyclohexyl acetate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% "Verdox ®" in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15)

Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010). A RIFM review is available (58).

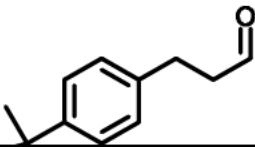
4-TERT-BUTYLCYCLOHEXYL ACETATE	
CAS # 32210-23-4	
EC # 250-954-9	
4-(1,1-Dimethylethyl)cyclohexyl acetate	
Boisinal A 464D; Cyclohexanol, 4-tert-Butyl-, acetate; Cyclohexanol, 4-(1,1-Dimethylethyl)-, acetate; 4-(1,1-Dimethylethyl)cyclohexyl acetate; 4-tert-Butylcyclohexanol acetate; Dorisyl; Madeflor; NSC 163103; Oryclone, Oryclone special, Oryclon extra; p-t-BCHA; p-tert-Butylcyclohexyl acetate; para-tert-Butylcyclohexyl acetate; PTBCHA; Velvetone; Verbeniax; Vertenex; Vertinate; Vertopol; Ylanate	

Current regulation: /

Clinical data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% "Vertenex ®" in pet., tested in 107 consecutive patients in High Wycombe, were observed (15).

Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010). A RIFM review is available (59).

p-tert -Butyldihydrocinnamaldehyde	
CAS # 18127-01-0	
EC # 242-016-2	

4-(1,1-Dimethylethyl)-benzenepropanal	
p-tert-Butyl-hydrocinnamaldehyde; 3-(4-tert-Butylphenyl)propanal; Bourgeonal; p-tert-Butyldihydrocinnamaldehyde	

Current regulation: III/155

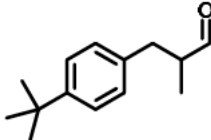
Clinical data: /

Additional

information:

It is a "top 200" substance and classified as R43 (IFRA, pers. comm.2010)

http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=39132

BUTYLPHENYL METHYLPROPIONAL (Lilial®)	
CAS # 80-54 6	
EC # 201-289-8	
3 (4 tert Butylphenyl)-2-methylpropanal	
<p>p-t-Butyl-α-methylhydrocinnamic aldehyde; 2-(4-tert-Butylbenzyl)propionaldehyde (REACH, EINECS); 4-(1,1-Dimethylethyl)-α-methyl-benzenepropanal; Hydrocinnamaldehyde, p-tert-Butyl-α-methyl-; (\pm)-2-Methyl-3-(4-tert-butylphenyl)propanal; 2-Methyl-3-(4-tert-butylphenyl)propanal; 2-[(4-tert-Butylphenyl)methyl]propanal; 3-(4-tert-Butylphenyl)-2-methylpropanal; 3-(p-tert-Butylphenyl)-2-methylpropionaldehyde; 3-(p-tert-Butylphenyl)isobutylaldehyde; 4-(1,1-Dimethylethyl)-α-methylbenzenepropanal; 4-tert-Butyl-α-methylhydrocinnamic aldehyde; Lilestralis; Lilial; Lysmeral; NSC 22275; lilestral; p-tert-Butyl-α-methylhydrocinnamaldehyde; p-tert-Butyl-α-methylhydrocinnamic aldehyde; pt-Bucinal; α-Methyl-p-tert-butylhydrocinnamaldehyde; β-Lilial</p>	

Current regulation: Annex III, part 1, n° 83

Clinical data:

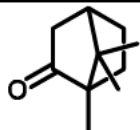
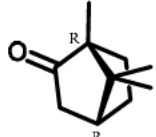
In the "background information" section of the 1999 opinion, lilial is classified as "less frequently reported allergen"; with 2 cases of contact allergy reported in 1 study of 176 eczema patients and 1 case with contact allergy to Lilial from a deodorant; a number of other reported positive cases were considered to possibly have been false positive (33).

Since the last SCCNFP-opinion of 1999, the Frosch 2002a study yielded 0.2% positive reactions to Lilial® (10% pet.) among the 1855 consecutive patients tested (16). The IVDK 2007 study yielded 0.4% (95% CI: 0.2 – 0.8%) positive reactions in 2004 patients consecutively tested (4). The IVDK 2010 study, 0.62% (95% CI: 0.04 – 1.21%; percentages standardised for age and sex) of 1947 patients PTed reacted to the compound (7). In the Groningen 2009 study, n=2, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at only 1% pet. (6). In the deGroot 2000 study, 9 of 1825 consecutively tested patients had a positive reaction to lilial® (5%

pet.) (12). Lilial® has been identified as constituent of perfumes used by a patient, causing ACD (60).

Additional information:

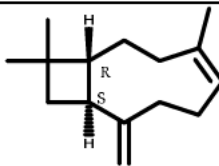
It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

CAMPHOR	 76-22-2
CAS # 76-22-2 / 464-49-3	
EC # 207-355-2 / 200-945-0	
1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one (76-22-2) (1R,4R)-1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one (464-49-3)	 464-49-3
76-22-2: DL-Bornan-2-one (REACH, EINECS); 2-Bornanone; Bornan-2-one, INCI name according to CAS; CAMPHOR/DL-bornan-2-one; Camphor; (±)-Camphor; DL-Camphor; 1,7,7-Trimethylnorcamphor; 2-Camphanone; Alphanon; Borneo camphor; Root bark oil; Spirit of camphor 464-49-3: (1R)-1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one; (1R,4R)-(+)-Camphor; (+)-2-Bornanone; (+)-Camphor; (1R)-(+)-Camphor; (1R)-Camphor; (1R,4R)-(+)-Camphor; (R)-(+)-Camphor; (R)-Camphor; Camphor; D-Camphor; D-(+)-Camphor; Alcanfor; Japanese camphor.	

Current regulation: /

Clinical data:
 From the UK, a case of allergic contact dermatitis after application of Earex ® ear drops due to rectified camphor oil (tested 10% pet.) was reported (61). Application of a liquid rubefacient of Asian origin caused allergic contact dermatitis in a 58-year-old patient, according to the positive PT result with 10% camphor ("alcaonfor") in pet. due to this ingredient (62). In the US, a case of contact dermatitis due to "Vics VapoRub" has been reported (63).

Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010).

<i>beta</i>-CARYOPHYLLENE	
CAS # 87-44-5	
EC # 201-746-1	
(1R,4E,9S)-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene	
(E)-(1R,9S)-(-)-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene; [1R-(1R*,4E,9S*)]-4,11,11-Trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene; (-)-(E)-Caryophyllene; (-)-Caryophyllene; (-)-E-Caryophyllene; (-)-trans-Caryophyllene; (-)-β-Caryophyllene; (E)-Caryophyllene; Caryophyllene; Caryophyllene B; NSC 11906; l-Caryophyllene; trans-Caryophyllene; β-Caryophyllen; β-Caryophyllene; (-)-β-Caryophyllene	

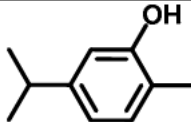
Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.6% positive reactions to caryophyllene (5% pet.) in 1606 consecutive were observed (17).

Additional information:

beta-Caryophyllene autoxidizes at air exposure. As the primary oxidation products, the hydroperoxides, are very unstable and immediately form epoxides with low sensitizing capacity, the increase in allergenic activity caused by autoxidation is comparably low (64). A multicenter study identified 0.5% positive reactions to oxidized *beta*-caryophyllene (3.0% pet.) in 1511 consecutive patients (65). Of these, 2 patients (0.1%) reacted to the major oxidation product (caryophyllene oxide) (3.9% pet.).

CARVACROL	
CAS # 499-75-2	
EC # 207-889-6	
2-Methyl-5-(1-methylethyl)-phenol	
2-Hydroxy-1-methyl-4-(1-methylethyl)benzene; 2-Hydroxy-p-cymene; 2-Methyl-5-(1-methylethyl)phenol; 2-Methyl-5-isopropylphenol; 3-Isopropyl-6-methylphenol; 5-Isopropyl-2-methylphenol; 5-Isopropyl-o-cresol; 6-Methyl-3-isopropylphenol; Antioxine; Dentol; Isopropyl o-cresol; Isothymol; NSC 6188; p-Cymen-2-ol	

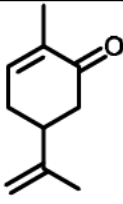
Current regulation: /

Clinical data:

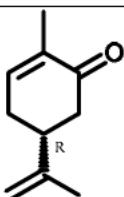
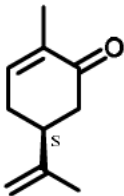
The DeGroot 1985 study identified 2 (1.1%) positive reactions among 179 patients using a 5% PT preparation of this compound – these reactions may have been at least partly due to an “excited back syndrome” and are thus of limited evidence (25). Meynadier et al. ¹¹ patch tested 28 patients with contact allergy to fragrance ingredients using 2% carvacrol in pet. Positive reactions were observed in 3 of 28 patients (after (66)).

Additional information:

Carvacrol is derived from p-cymene by sulfonation followed by alkali fusion. Carvacrol can also be derived from savory, thyme, marjoram, oregano, lovage root, and Spanish origanum oil (66). Carvacrol is a flavor ingredient that can be found in alcoholic beverages, baked goods, chewing gum, condiment relish, frozen dairy, gelatin pudding, non-alcoholic beverages, and soft candy at concentrations from 0.1 to 28.54 ppm (RIFM 2001, according to (66)).

CARVONE	
CAS # 99-49-0 / 6485-40-1 / 2244-16-8	
EC # 202-759-5 / 229-352-5 / 218-827-2	
2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (99-49-0)	

¹¹ Meynadier, J. M., J. Meynadier, J. L. Peyron, and L. Peyron. 1986. Clinical forms of skin manifestations in allergy to perfume. *Ann. Dermatol. Venerol.* 113:31–39.

(5R)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (6485-40-1)	 6485-40-1	
(5S)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (2244-16-8)		
99-49-0: p-Mentha-6,8-dien-2-one; (±)-Carvone; 2-Methyl-5-isopropenyl-2-cyclohexenone; 5-Isopropyl-2-methyl-2-cyclohexen-1-one; Carvone; DL-Carvone; Karvon; Limonen-6-one; NSC 6275; p-Mentha-1(6),8-dien-2-one	 2244-16-8	
6485-40-1: R)-(-)-p-Mentha-6,8-dien-2-on); (-)-(5R)-Carvone; (-)-(R)-Carvone; (-)-Carvone; (-)-p-Mentha-6,8-dien-2-one; (4R)-(-)-Carvone; (R)-(-)-Carvone; (R)-Carvone; L-(-)-Carvone; L-Carvone; l-1-Methyl-4-isopropenyl-6-cyclohexen-2-one; l-Carvone		
2244-16-8: (S)-(+)-p-Mentha-6,8-dien-2-one; (+)-Carvone; (S)-(+)-Carvone; (S)-(+)-p-Mentha-6,8-dien-2-one; (S)-Carvone; (+)-Carvone; D-(+)-Carvone; D-Carvone; Talent; d-1-Methyl-4-isopropenyl-6-cyclohexen-2-one; (S)-2-Methyl-5-(1-methylvinyl)cyclohex-2-en-1-one; d-Carvone		

Current regulation: /

Clinical data:

Cases of allergic contact cheilitis due to L-carvone in toothpastes have been reported (67-69). In an earlier study, 15 of 541 (2.8%) of consecutive PT patients tested also with L-Carvone (5% pet.) exhibited positive reactions, which were (i) associated with positive PT results to *Compositae* mix and (ii) mostly were not considered clinically relevant. Upon re-testing with lower concentrations (2% and 1% pet.) only 2 of 8 patients thus tested were positive (70).

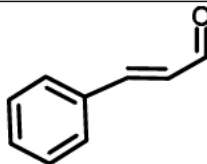
"Carvone has occasionally been reported as an allergen, usually in flavourings. Isomers of carvone have been either a mint or a rye flavour and aroma. We report a woman with positive patch-test reactions to carvone (newly added to the North American Contact Dermatitis Group standard series) and dermatitis on the head. She had used a hair conditioner with a "mint" scent, and the dermatitis resolved when she discontinued using this product. While the manufacturer would not confirm carvone as an ingredient, the clinical course, patch-test results, and ingredient list strongly suggest that this was a relevant allergen in this case of allergic contact dermatitis"¹²

Additional information:

D-Carvone occurs in caraway seed oil and dill oil in a concentration of up to 60%. L-Carvone is a component of the oil from *Mentha spicata* (spearmint).

R-Carvone is identified as a secondary oxidation product in autoxidized limonene (71). However, it is not a major allergen in this oxidation mixture and only one of 30 patients with known contact allergy to oxidized R- limonene reacted when tested with carvone (3% pet.) (72). Experimental findings in guinea pigs show no cross reactivity between R- and S carvone, but both enantiomers were found to be equally strong sensitizers (73).

¹² <http://www.ncbi.nlm.nih.gov/pubmed/20233552>

CINNAMAL	
CAS # 104-55-2	
EC # 203-213-9	
3-Phenyl-2-propenal	
Cinnamaldehyde; 3-Phenyl-2-propen-1-al; 3-Phenyl-2-propenaldehyde; 3-Phenylacrolein; 3-Phenylacrylaldehyde; 3-Phenylpropenal; Abion CA; Benzylideneacetaldehyde; Cassia aldehyde; Cinnacure; Cinnamal; Cinnamic aldehyde; Cinnamite; Cinnamyl aldehyde; NSC 16935; NSC 40346; Phenylacrolein; Zimtaldehyde; β -Phenylacrolein	

Current regulation: Annex III, part 1, n° 76

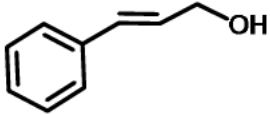
Clinical data:
In the "background information" section of the previous opinion (33), cinnamal, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products in several studies (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.0% (95% CI: 0.6 – 1.6%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 1.6% (95% CI: 0.5 – 3.6%) had positive reactions to cinnamal (6). In a study by the North American Contact Dermatitis Group, no significant trend of cinnamal contact sensitisation in the consecutive patients analysed was observed between 1984 (5.9% pos.) and 2000 (3.6% pos.); tested at 1% pet. (74). In the An 2005 study, 7 of 422 consecutive patients, i.e., 1.7%, had positive reaction (13). The Belsito 2006 study (20) yielded 1.7% positive reactions. In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.9% positive reactions (22). The NACDG study found 3.1% positive reactions in 4435 patients tested (21). The IVDK 2010 study, 1.43% (95% CI: 0.67 – 2.18%) of 1214 consecutively tested patients reacted to the compound, while 2.64% (95% CI: 2.16 – 3.13%) of 4527 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=7 reacted positively to cinnamal (48).

While, in addition to typical ACD due to contact sensitisation, immediate reactions to some fragrance compounds (and MPR, see below) are observed not infrequently, such immediate type reactions may rarely be very severe (anaphylaxis) and possibly immunologically mediated, as illustrated by the case of a 42 year old nurse with anaphylaxis (maximum grade of contact urticaria syndrome) 20 min after application of cinnamal (75). Following industrial use as "odour masking" agent, cinnamal caused occupational ACD in an exposed worker (76).

Additional information:

A specific RIFM review is available (77); another RIFM review addresses several cinnamic compounds (78).

CINNAMYL ALCOHOL	
CAS # 104-54-1	
EC # 203-212-3	
3-Phenyl-2-propen-1-ol	

Cinnamyl alcohol; 1-Phenyl-3-hydroxy-1-propene; 1-Phenylprop-1-en-3-ol; 3-Hydroxy-1-phenylprop-1-ene; 3-Phenyl-2-propenol; 3-Phenylallyl alcohol; Cinnamic alcohol; NSC 623440; NSC 8775; Styrene; Styryl alcohol; Styryl carbinol; γ -Phenylallyl alcohol	
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Current regulation: Annex III, part 1, n° 69

Clinical data:

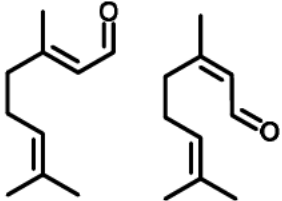
In the “background information” section of the previous opinion (33), cinnamyl alcohol, one of the 8 constituents of the FM I, is classified as frequent allergen, causing allergic reactions in a notable persons with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.6% (95% CI: 0.3 – 1.1%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 2.5% (95% CI: 1.1 – 4.9%) had positive reactions to cinnamyl alcohol, tested at 2% pet., i.e., twice the commonly used concentration (6). As test concentrations of up to 5% are apparently non-irritating (de Groot et al. after (33)), the latter data can be regarded as valid. In the An 2005 study, 13 of 422 consecutive patients, i.e., 3.1%, had positive reaction (13) (test concentration 2%). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.5% positive reactions (22). The IVDK 2010 study, 0.73% (95% CI: 0.17 – 1.30%) of 1214 consecutively tested patients reacted to the compound, while 2.36% (95% CI: 1.89 – 2.83%) of 4502 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to cinnamyl alcohol (48).

Additional information:

In a recent experimental study protein-cinnamal adducts were detected in skin homogenates treated with cinnamal and cinnamyl alcohol but not with alpha-amyl cinnamal. This suggests that there is a common hapten involved in cinnamal and cinnamyl alcohol sensitization, in line with the observation of a marked concordance upon patch testing (7, 79), and that metabolic activation (to cinnamal) is involved in the latter. Conversely, there does not appear to be a common hapten for cinnamal and alpha-amyl cinnamal (80), again in line with the observations in the IVDK 2010 study (7).

A RIFM review is available (81)

CITRAL	
CAS # 5392-40-5	
EC # 226-394-6	
3,7-Dimethyl-2,6-octadienal	
3,7-Dimethyl-2,6-octadien-1-al; Citral; Citral PQ Extra; Lemarome N; Lemsyn GB; NSC 6170	<p>Citral = isomeric mixture of Geranial and Neral</p>

Current regulation: Annex III, part 1, n° 70

Clinical data:

In the “background information” section of the previous opinion (33), citral is classified as frequent allergen, causing about 1% allergic reactions in consecutive PT patients, and being a proven cause of contact allergic reactions in 2.6% patients with eczema from

cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the Frosch 2002 a study yielded 1.1% positive (and 1.3% doubtful) reactions among the 1855 consecutive patients tested (16). In a study on 586 consecutive patients with hand eczema it has been noted that citral (2% pet.) not only caused (mostly weak) positive PT reactions, but far more often irritant reactions (n=82 vs. n=28). It was hypothesised that this very property could contribute to citral's sensitising potential (82). In the EU 2005 study, 12 of 1701 patients (0.7%, 95% CI: 0.4 – 1.2%) reacted positively to 2% citral in pet. (10). The IVDK 2007 study yielded 0.6% (95% CI: 0.3 – 1.1%) positive reactions in 2021 consecutively PTed patients; 10 of 13 citral positive patients also reacted positively to geraniol (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6). In the deGroot 2000 multicentre study, 19 of 1825 consecutive patients tested positively to citral (2% pet.), 4 of whom did not react positively to the FM I (12). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reaction (13) (test concentration 2%). In the Malten 1984 study, neral at 1% in pet. yielded 2.6% positive reactions in 182 patients (24). In a study from Alicante, Spain, 86 selected patients were tested with citral, yielding 2 positive reactions (48).

Citral in a lip salve has been reported to have caused longstanding, recurrent allergic contact cheilitis in a 30 year old female patient, diagnosed by a strong positive reaction to the FM II, followed by a strong positive reaction to citral (83).

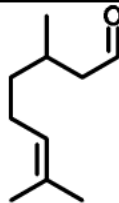
Additional information:

Citral is the mixture of two isomers: cis-citral (neral) and trans-citral (geranial).

Geranial forms oxidation product with increased sensitizing capacity both via spontaneous autoxidization at air exposure and via metabolic oxidation (Hagvall L. Thesis 2009: <http://hdl.handle.net/2077/18951>).

Geranial and neral have been identified as secondary oxidation products when geraniol autoxidizes (84). They have also been identified as metabolites of geraniol (85). This explains the simultaneous reactions to geraniol and citral seen by (4).

It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

CITRONELLAL	
CAS # 106-23-0	
EC # 203-376-6	
3,7-Dimethyl-6-octenal	
(±)-Citronellal; 2,3-Dihydrocitral; 3,7-Dimethyloct-6-en-1-al; Citronellal; NSC 46106; Rhodinal; dl-Citronellal; β-Citronellal	
Current regulation: /	
Clinical /	data:
Additional information: A compound of essential oils of citrus fruits, namely grapefruit, but also contained in "citronella oil" and oil of Melissa.	

CITRONELLOL	 106-22-9	
CAS # 106-22-9 / 1117-61-9 / 7540-51-4	 1117-61-9	
EC # 247-737-6 / 214-250-5 / 231-415-7	 7540-51-4	
3,7-Dimethyl-6-octen-1-ol (106-22-9); (3R)-3,7-Dimethyl-6-octen-1-ol (1117-61-9); (3S)-3,7-Dimethyl-6-octen-1-ol (7540-51-4)		
106-22-9: (±)-3,7-Dimethyl-6-octen-1-ol; (±)-Citronellol; (±)-β-Citronellol; 2,3-Dihydrogeraniol; 2,6-Dimethyl-2-octen-8-ol; Cephrol; Citronellol; Citronellol 950; DL-Citronellol; Dihydrogeraniol; NSC 8779; Rodinol; dl-Citronellol; β-Citronellol		
1117-61-9: (R)-3,7-Dimethyl-6-octen-1-ol; (R)-(+)-3,7-Dimethyl-6-octen-1-ol; (+)-(R)-Citronellol; (+)-Citronellol; (+)-β-Citronellol; (3R)-(+)-β-Citronellol; (R)-(+)-Citronellol; (R)-(+)-β-Citronellol; (R)-Citronellol; (R)-β-Citronellol; D-Citronellol; d-Citronellol		
7540-51-4: (-)-3,7-Dimethyl-6-octen-1-ol; (-)-(S)-Citronellol; (-)-Citronellol; (-)-β-Citronellol; (S)-(-)-Citronellol; (S)-(-)-β-Citronellol; (S)-3,7-Dimethyl-6-octen-1-ol; (S)-Citronellol; (S)-β-Citronellol; L-Citronellol; l-Citronellol		

Current regulation: Annex III, part 1, n° 86

Clinical data:

In the "background information" section of the 1999 opinion, citronellol is classified as "less frequently reported allergen"; with few cases of contact allergy reported in the literature (33).

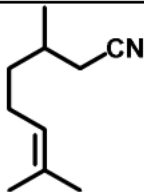
Since the last SCCNFP-opinion of 1999, in the Larsen 2002 c study, „DL citronellol“ (5% in pet.) elicited positive PT reactions in 8.7% of the patients (1). In 1855 consecutive patients of the Frosch 2002 a study, 0.4% positive reactions were noted (16). In the EU 2005 study, 4 of 1701 patients (0.2%, 95% CI: 0.06 – 0.6%) reacted positively to 1%

citronellol in pet.; at the same concentration, n=23 doubtful or irritant reactions were observed (10). The IVDK 2007 study yielded 0.5% (95% CI: 0.2 – 0.9%) positive reactions in 2003 patients consecutively PTed (4). In the Groningen 2009 study, n=1, i.e. 0.3% (95% CI: 0.01 – 1.7%) had positive reactions to this allergen, tested at only 2% pet. (6). The Larsen 2001 study yielded 5.6% positive reactions to l-citronellol (5% pet.) in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information:

Citronellol autoxidizes spontaneously in contact with air in the same way as linalool forming allergenic primary oxidation products, hydroperoxides (AT Karlberg, personal communication, 2011).

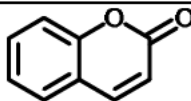
RIFM reviews have been published regarding L-citronellol (86), D-citronellol (87) and DL-citronellol (88). Another review is available by Hostynek and Maibach (89). It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

CITRONELLYL NITRILE	
CAS # 51566-62-2	
EC # 257-288-8	
3,7-Dimethyl-6-octenenitrile	
3,7-Dimethyl-6-octenenitrile (REACH, EINECS, INCI); Agrunitril; Agrunitrile; Citronellyl nitrile	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010)

COUMARIN	
CAS # 91-64-5	
EC # 202-086-7	
2H-1-Benzopyran-2-one	
1,2-Benzopyrone; 2-Chromenone; 2-Propenoic acid, 3-(2-hydroxyphenyl)-, δ-lactone; 5,6-Benzo-2-pyrone; Benzo-α-pyrone; Coumarinic anhydride; NSC 8774; Rattex; Tonka bean camphor; cis-o-Coumarinic acid lactone; o-Hydroxycinnamic acid lactone	

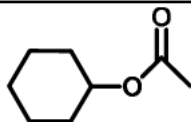
Current regulation: Annex III, part1, n° 77

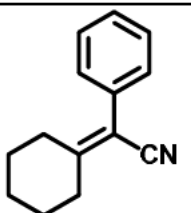
Clinical data:


In the "background information" section of the previous opinion (33), coumarin is classified as frequent allergen, causing allergic reactions in about 0.4 – 0.8% in consecutive PT patients, and causing contact allergic reactions in 0.8-10% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 0.3% positive PT reactions to consecutive patients were noted (16). In the EU 2005 study, none of the

methylpropionaldehyde; methylpropionaldehyde(REACH, EINECS); methylhydrocinnamic aldehyde; Cyclamal; Cyclamen aldehyde; Cyclosal; Cyclosal perfume; Cymal; p-Isopropyl- α - methylhydrocinnamaldehyde; methylethyl)benzenepropanal; isopropylhydrocinnamaldehyde	3-p-Cumenyl-2- 4-Isopropyl- α - α -Methyl-4-(1- α -Methyl-p-	
Current regulation: ...		
Clinical /		data:
Additional information: It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).		

CYCLOHEXYL ACETATE	
CAS # 622-45-7	
EC # 210-736-6	
Cyclohexyletanoat	
Acetic acid cyclohexanyl ester; Acetoxycyclohexane; Cyclohexyl acetate; NSC 8772	
Current regulation: /	
Clinical data:	
In the Larsen 2002 c study, 0.5% positive reactions among 218 patients with known contact allergy to fragrance ingredients were observed (1).	
Additional information: A RIFM review is available (92).	

<i>alpha</i>-CYCLOHEXYLIDENE BENZENEACETONITRILE	
CAS # 10461-98-0	
EC # 423-740-1	
α-Cyclohexylidenebenzeneacetonitrile	
alpha-Cyclohexylidene-benzeneacetonitrile (REACH); Δ 1 α -Phenyl- α -Cyclohexaneacetonitrile; 2-Cyclohexylidene-2-phenylacetonitrile; NSC 408284; Peonile (REACH)	
Current regulation: /	
Clinical data: /	
Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).	

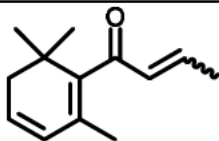
CYCLOPENTADECANONE	
CAS # 502-72-7	

EC # 207-951-2	
Cyclopentadecanone	
CPE 218; Exaltone; NSC 63900; Normuscon; Normuscone	

Current regulation: /

Clinical data:
In the Larsen 2001 study, n=3, i.e., 1.7% positive reactions were observed to the compound, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information: ...

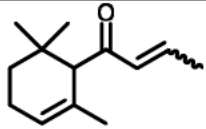
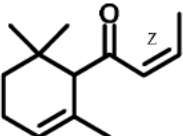
DAMASCENONE	
ROSE KETONE-4 (Not officially an INCI Name but Perfuming Name; Damascenone as such is not listed in CosIng)	
CAS # 23696-85-7	
EC # 245-833-2	
1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one	
1-(2,6,6-Trimethyl-1,3-cyclohexadienyl)-2-buten-1-one; 1-Crotonoyl-2,6,6-trimethyl-1,3-cyclohexadiene; 2,6,6-Trimethyl-1-(2-butenoyl)-1,3-cyclohexadiene; 2,6,6-Trimethyl-1-crotonyl-1,3-cyclohexadiene; Rose ketone # 4	

Current regulation: Annex III, part1, n° 160 (max. conc. 0.02%)

Clinical data: /

Additional information:

RIFM reviews are available (93, 94), quoting 1 negative, and 2 positive (2 of 37, 1 of 50 volunteers) HRIPTs with damascenone based on 2 LLNA, the EC3 values were calculated as 1.24% and 1.22%, respectively (94).

<i>alpha-DAMASCONE (TMCHB)</i>	 43052-87-5
CAS # 43052-87-5 / 23726-94-5	
EC # x / 245-845-8	
1-(2,6,6-Trimethylcyclohex-2-en-1-yl)but-2-enone (43052-87-5); (2Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one (23726-94-5)	
43052-87-5: 2,6,6-Trimethyl-1-crotonyl-2-cyclohexene; α -Damascone	
23726-94-5: (Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one; (Z)- α -Damascone; cis- α -Damascone	 23726-94-5

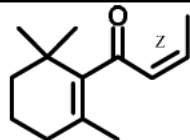
Current regulation: Annex III, part1, n° 157 (max. conc. 0.02%)

Clinical data:
In the Frosch 2002 b study, n=8 (0.5%) mostly strong positive PT reactions to consecutive patients were noted using a mixture of alpha and beta damascene, 0.1% pet. each (17). In human sensitisation experiments, after epicutaneous induction with 30% 1-(2,6,6-trimethylcyclohex-2-en-1-yl)but-2-enone (TMCHB, CAS # 43052-87-5) with adjuvant, to enhance response to this weak sensitiser, 8 of 30 patients were elicited by a challenge with 3% TMCHB 2 weeks later (95).

Additional information:

The former CAS # refers to alpha-Damascone or 1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-Buten-1-one. The latter CAS # refers to the identified ingredient cis-alpha-Damascone or (Z)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one, the content of which is restricted (SCCS-opinion 0392/00).

A RIFM review is available on alpha-damascone (96), quoting a number of partly positive HRIPT and other human studies, as well as different animal experiments. In 1 LLNA reported, an EC3 value of 3.3% was found. Another RIFM review is available for cis-alpha-damascone (97), supplying, however, no data on sensitisation.

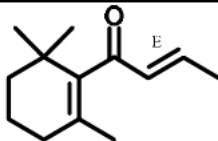
<i>cis-beta-DAMASCONE</i>	
CAS # 23726-92-3	
EC # 245-843-7	
(2Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	
(Z)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (Z)- β -Damascone	

Current regulation: Annex III, part 1, n° 162 (max. conc. 0.02%)

Clinical data:
Regarding results of the Frosch 2002 b study, see under alpha-damascone.

Additional information:

A RIFM review is available (98), citing several negative and one positive HRIPTs, and a number of – mostly positive – animal experiments.

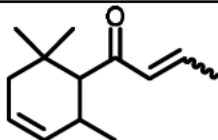
<i>trans-beta-DAMASCONE</i>	
CAS # 23726-91-2	
EC # 245-842-1	
(2E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one	
(E)-1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-buten-1-one; (E)- β -Damascone; Damascone beta; trans-2,6,6-Trimethyl-1-crotonylcyclohex-1-ene; trans- β -Damascone; β -Damascone	
Current regulation: Annex III, part 1, n° 158 (max. conc. 0.02%)	

Current regulation: Annex III, part 1, n° 158 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (99), citing 2 negative HRIPT and 1 negative maximisation test, and a number of positive animal experiments (the EC3 value, based on 1 LLNA, was found to be 2.4%).

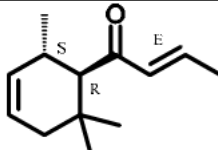
<i>delta-DAMASCONE</i>	
CAS # 57378-68-4	
EC # 260-709-8	
1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one	
δ -Damascone	

Current regulation: Annex III, part 1, n° 161 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (100), citing several positive HRIPT and 1 negative HRIPT. Cross sensitisation to alpha- and beta-damascone was demonstrated in 3 sensitised subjects. 2 LLNA studies are reported on, yielding EC3 values of 5.19% and 9.6%, resp.

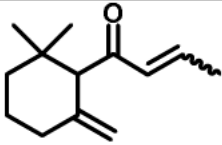
<i>trans-trans-delta-DAMASCONE</i>	
CAS # 71048-82-3	
EC # 275-156-8	
(2E)-rel-1-[(1R,2S)-2,6,6-Trimethyl-3-cyclohexen-1-yl]- 2-buten-1-one	
[1 α (E),2 β]-1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one; trans- δ -Damascone; δ -Damascone; trans, trans- δ -Damascone	

Current regulation: Annex III, part 1, n° 165 (max. conc. 0.02%)

Clinical data: /

Additional information:

A RIFM review is available (101), citing 1 positive HRIPT (2/15 with 1%).

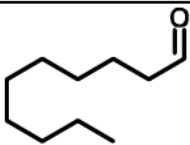
<i>gamma-DAMASCONE</i>	
CAS # 35087-49-1	
EC # 481-910-9	
1-(2,2-Dimethyl-6-methylenecyclohexyl)-2-buten-1-one	
γ-Damascone	

Current regulation: /

Clinical data: /

Additional information:

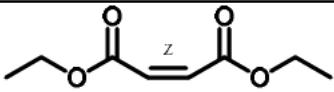
A RIFM review is available (102), citing 1 positive Buehler test and 1 LLNA study yielding an EC3 value of 4.6%

<i>DECANAL</i>	
CAS # 112-31-2	
EC # 203-957-4	
n-Decanal	
Capraldehyde; Capric aldehyde; Caprinaldehyde; Caprinic aldehyde; Decaldehyde; Decanaldehyde; Decyl aldehyde; Decylic aldehyde; NSC 6087; n-Decaldehyde; n-Decyl aldehyde	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

<i>DIETHYL MALEATE</i>	
CAS # 141-05-9	
EC # 205-451-9	
(2Z)-Diethyl but-2-enedioate	
2-Butenedioic acid (2Z)-, diethyl ester; 2-Butenedioic acid (Z)-, diethyl ester; Maleic acid, diethyl ester; (2Z)-2-Butenedioic acid diethyl ester; Diethyl (Z)-2-butenedioate; Ethyl maleate; Staflex DEM	

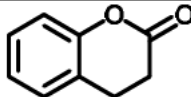
Current regulation: Annex II, n° 426

Clinical

data:

In the Malten 1984 study, 3.2% of 182 patients displayed a positive PT reaction to diethyl maleate 0.1% pet. (24). In this study, it has been noted that "in the max. test and clinically this is a strong sensitiser having caused patch test sensitisation (42%)"

Additional information: /

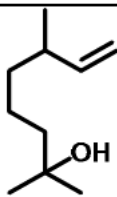
DIHYDROCOUMARIN	
CAS # 119-84-6	
EC # 204-354-9	
3,4-Dihydro-2H-1-benzopyran-2-one	
Hydrocoumarin; Hydrocinnamic acid, o-hydroxy-, δ -lactone; 2-Chromanone; 3,4-Dihydro-1H-benzopyran-2-one; 3,4-Dihydrocoumarin; Dihydrocoumarin; Melilotin; Melilotin (coumarin); Melilotol	

Current regulation: Annex II, n° 427

Clinical data:

In the Malten 1984 study, 3.7% of 182 patients displayed a positive PT reaction to dihydrocoumarine 5% pet. (24).

Additional information: /

DIHYDROMYRCENOL	
CAS # 18479-58-8	
EC # 242-362-4	
(±)-2,6-Dimethyloct-7-en-2-ol	
1,1,5-Trimethyl-6-heptenol; 2,6-Dimethyl-7-octen-2-ol; 3,7-Dimethyl-1-octen-7-ol; 2,6-Dimethyl-7-octen-2-ol (INCI)	

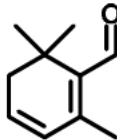
Current regulation: /

Clinical data: /

Additional information:

A RIFM review is available (103), listing 2 negative HRIPTs and 1 negative human maximisation test.

It is a "top 100" substance (IFRA, pers. comm.2010).

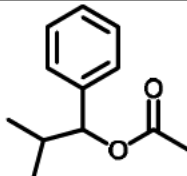
2,3-DIHYDRO-2,2,6-TRIMETHYLBENZALDEHYDE	
CAS # 116-26-7	
EC # 204-133-7	
2,6,6-Trimethyl-1,3-cyclohexadiene-1-carboxaldehyde	
2,2,6-Trimethyl-4,6-cyclohexadien-1-aldehyde; 2,6,6-Trimethyl-1,3-cyclohexadiene-1-aldehyde; Safranal	

Current regulation: /

Clinical data: /

Additional information:

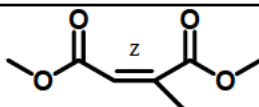
A RIFM review quotes one positive HRIPT (5 of 53) and one negative HRIPT (0 of 54) (93).

DIMETHYLBENZYL CARBINYL ACETATE (DMBCA)	
CAS # 151-05-3	
EC # 205-781-3	
2-Methyl-1-phenylpropyl acetate	
Benzeneethanol, α,α-dimethyl-, acetate; Phenethyl alcohol, α,α-dimethyl-, acetate; 1,1-Dimethyl-2-phenylethyl acetate; 2-Methyl-1-phenyl-2-propyl acetate; 2-Methyl-1-phenylpropan-2-yl acetate; Benzyl dimethylcarbinol acetate; Benzyl dimethylcarbinyl acetate; Dimethylbenzylcarbinol acetate; Dimethylbenzylcarbonyl acetate; NSC 46123; α,α-Dimethylphenethyl acetate	

Current regulation: /

Clinical data:
In the Frosch 2002 a study, 0.2% positive PT reactions to consecutive patients were noted (16). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and one to 5% DMBCA in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

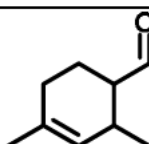
DIMETHYL CITRACONATE	
CAS # 617-54-9	
EC #	
(2Z)-Diethyl-2-metyl-but-2-enedioate	
(2Z)-2-methyl-2-Butenedioic acid, dimethyl ester; 2-Butenedioic acid, 2-methyl-, dimethyl ester, (Z)-; Citraconic acid, dimethyl ester; Dimethyl methylmaleate; Methylmaleic acid, dimethyl ester	

Current regulation: Annex II, n° 431

Clinical data:

In the Malten 1984 study, 3.7% of 182 patients displayed a positive PT reaction to dimethylcitraconate 12% pet. (24). In this paper, a human maximisation test positive in "4/44" is quoted.

Additional information: ...

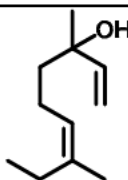
2,4-DIMETHYL-3-CYCLOHEXEN-1-CARBOXALDEHYDE	
CAS # 68039-49-6	
EC # 268-264-1	
2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde	
(Z)-Vertocitral C; 2,4-Dimethyl-3-cyclohexene-1-carboxaldehyde; 2,4-Dimethyl-3-cyclohexenecarboxaldehyde; 2,4-Dimethyl-3-cyclohexenylcarbaldehyde; Cyclal C; Ligustral; Tricyclal; Triplal; Tripral; Zestover	

Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

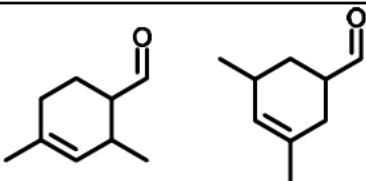
3,7-DIMETHYL-1,6-NONADIEN-3-OL	
CAS # 10339-55-6	
EC # 233-732-6	
(7Z)-3,7-Dimethyl-1,6-nonadien-3-ol	
Ethyl linalool; Methyl linalool	

Current regulation: /

Clinical data: /

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010). A RIFM review is available (104), citing 1 negative human maximisation test (n=25).

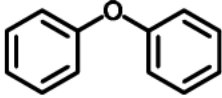
DIMETHYLTETRAHYDRO BENZALDEHYDE	
CAS # 68737-61-1	
EC # 272-113-5	
2,4-Dimethyl-cyclohex-3-ene-1-carboxaldehyde 3,5-Dimethyl-cyclohex-3-ene-1-carboxaldehyde	
Hivertal; Vertocitral	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 2.3% positive PT reactions were observed with the isomer mixture, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

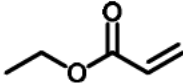
Additional information: /

DIPHENYL ETHER	
CAS # 101-84-8	
EC # 202-981-2	
Phenyl ether	
1,1'-oxybis-Benzene; Barrel Therm 330; Benzene, phenoxy-; Biphenyl oxide; Chemcryn JK-EB; Diphenyl ether; Diphenyl oxide; NSC 19311; Oxybisbenzene; Phenoxybenzene; Phenyl oxide	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

ETHYL ACRYLATE	
CAS # 140-88-5	
EC # 205-438-8	
Ethyl 2-propenoate	
Acrylic acid ethyl ester (6CI,8CI); 2-Propenoic acid ethyl ester; Ethyl 2-propenoate; Ethyl acrylate; Ethyl acrylic ester; Ethyl propenoate; NSC 8263	

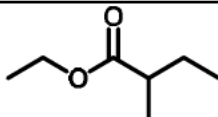
Current regulation: Annex II, n° 435

Clinical data:

In the Malten 1984 study, n=1 (0.5%) of 182 patients displayed a positive PT reaction to ethyl acrylate 1% pet. (24). In the NACDG 2009 multicentre study, 0.9% of

consecutive patients (n=4428) had a positive PT reaction (21).

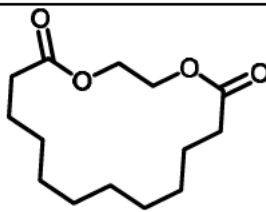
Additional information: /

ETHYL 2-METHYLBUTYRATE	
CAS # 7452-79-1	
EC # 231-225-4	
Ethyl 2-methylbutyrate	
Butyric acid, 2-methyl-, ethyl ester (6CI,7CI,8CI); (±)-Ethyl 2-methylbutanoate; 2-Methylbutanoic acid ethyl ester; 2-Methylbutyric acid ethyl ester; Ethyl 2-methylbutanoate; Ethyl 2-methylbutyrate; Ethyl α-methylbutyrate; NSC 1103	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

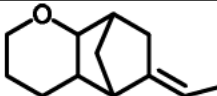
ETHYLENE DODECANEDIOATE	
CAS # 54982-83-1	
EC # 259-423-6	
1,4-Dioxacyclohexadecane-5,16-dione	
Cyclic ethylene dodecanedioate; Ethylene dodecanedioate; Musk 144; Musk C-14	

Current regulation: /

Clinical data:

In the Larsen 2002 c study on 218 patients with known contact allergy to fragrance ingredients, this compound caused 0.9% positive PT reactions at 5% pet. (1).

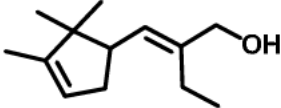
Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

6-ETHYLIDENEOCTAHYDRO-5,8-METHANO-2H-BENZO-1-PYRAN	
CAS # 93939-86-7	
EC # 300-376-9	
6-Ethylideneoctahydro-5,8-methano-2H-1-benzopyran	

Current regulation: /

Clinical data:
In the Larsen 2001 study, no positive PT reactions were observed with this compound, tested 5% pet., in 178 patients with known contact allergy to fragrance ingredients (19).

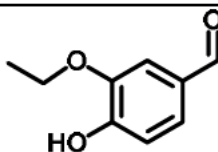
Additional information: /

2-ETHYL-4-(2,2,3-TRIMETHYL-3-CYCLOPENTEN-1-YL)-2-BUTEN-1-OL	
CAS # 28219-61-6	
EC # 248-908-8	
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol	
2-Ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-2-buten-1-ol; 2-Ethyl-4-(2',2',3-trimethylcyclopent-3'-enyl)but-2-enol; Bacdanol; Bangalol; Dartanol; Finanol; Levosandol; Radjanol; Sanjinol	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

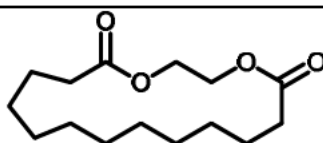
ETHYL VANILLIN	
CAS # 121-32-4	
EC # 204-464-7	
3-Ethoxy-4-hydroxybenzaldehyde	
2-Ethoxy-4-formylphenol; 3-Ethylvanillin; 4-Hydroxy-3-ethoxybenzaldehyde; Arovanillon; Bourbonal; Ethavan; Ethovan; Ethylprotal; Ethylvanillin; NSC 1803; NSC 67240; Protocatechuic aldehyde ethyl ether; Quantrovanil; Rhodiarome; Vanillal; Vanirom	

Current regulation: /

Clinical data:

The case of a 28-year-old metal grinder with allergic contact dermatitis to a "cutting oil reodorant" has been reported, who tested positively not only to the cutting fluid, the reodorant, but also to several ingredients of the latter product, including "Vanillal S 10026", 5% pet. (105).

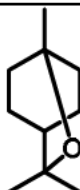
Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

ETHYLENE BRASSYLATE	
CAS # 105-95-3	
EC # 203-347-8	
1,4-Dioxacycloheptadecane-5,17-dione	
Tridecanedioic acid, cyclic ethylene ester; Ethylene glycol, cyclic tridecanedioate; Astratone; Cyclic ethylene glycol tridecanedioate; Cyclic ethylene tridecanedioate; Emeressence 1150; Ethylene brassylate; Musk T; NSC 46155	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

EUCALYPTOL	
CAS # 470-82-6	
EC # 207-431-5	
1,3,3-Trimethyl-2-Oxabicyclo[2.2.2]octane	
1,8-Epoxy-p-menthane; oxabicyclo[2.2.2]octane; 1,8-Cineol; 1,8-Cineole; 1,8-Epoxy-p-menthane; 2-Oxa-1,3,3-trimethylbicyclo[2.2.2]octane; Cajeputol; Cineol; Cineole; Eucalyptol; Eucalyptole; Eucalytol; Eucapur; Eukalyptol; NSC	

6171; Terpan; p-Cineole	
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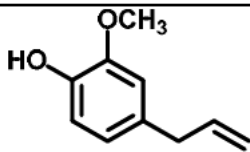
Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010).

See also **EUCALYPTUS SPP. LEAF OIL**; eucalyptol is the major ingredient there (up to 85%), but found in significant quantities also in a number of other essential oils (see 3.2).

EUGENOL	
CAS # 97-53-0	
EC # 202-589-1	
2-Methoxy-4-(2-propen-1-yl)-phenol	
Other names: 4-Allyl-2-methoxy-phenol; 1-Allyl-4-hydroxy-3-methoxybenzene; 2-Hydroxy-5-allylanisole; 2-Methoxy-1-hydroxy-4-allylbenzene; 2-Methoxy-4-(2-propenyl)phenol; 2-Methoxy-4-(2'-propenyl)phenol; 2-Methoxy-4-[2-allyl]phenol; 2-Methoxy-4-allylphenol; 3-(3-Methoxy-4-hydroxyphenyl)propene; 3-(4-Hydroxy-3-methoxyphenyl)-1-propene; 4-Allyl-1-hydroxy-2-methoxybenzene; 4-Allyl-2-methoxyphenol; 4-Allylguaiacol; 4-Hydroxy-3-methoxyallylbenzene; Allylguaiacol; Bioxeda; Caryophyllilic acid; Dentogum; Eugenilic acid; Eugenol; NSC 209525; NSC 8895; p-Allylguaiacol; p-Eugenol	

Current regulation: Annex III, part 1, n° 71

Clinical data:

In the "background information" section of the previous opinion (33), eugenol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 1.2% in consecutive PT patients and accounting for 4 to 16% of reactions to the FM I. Allergic reactions had been observed in 0.7 – 20% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.5% (95% CI: 0.3 – 1.0%) positive reactions in 2065 consecutively PTed patients (4). In the Groningen 2009 study, 1.3% (95% CI: 0.3 – 3.2%) had positive reactions to eugenol, tested at 2% pet., i.e., twice the commonly used concentration (6). F. Giusti et al. examined 1754 consecutive patients tested with eugenol 1% pet. in addition to the baseline series, 09/1998 - 01/2000. 21 patients (1.2%) reacted positively to eugenol (106). In the An 2005 study, 8 of 422 consecutive patients, i.e., 1.9%, had positive reaction (13) (test concentration 2%). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 2.5% positive reactions (22). The IVDK 2010 study, 0.44% (95% CI: 0.04 – 0.84%) of 1214 consecutively tested patients reacted to the compound, while 1.57% (95% CI: 1.19 – 1.95%) of 4801 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to eugenol (48).

Moreover, eugenol is capable of inducing immediate type reactions of the airways, as illustrated by the well-documented case of a 30 year old hairdresser who developed severe occupational bronchial asthma due to eugenol (107). A case of urticaria after dental treatment with eugenol-containing material was reported from India (108); however, occasional cases are also reported from Europe (109). Occupational exposure to eugenol / zinc oxide type dental restorative material, which is apparently less frequently used nowadays, may lead to occupational sensitisation to eugenol, as illustrated by a case report (110).

Additional information:

Eugenol is the main component (80-95%) of clove oil, but also found in citronella oil, pimento leaf oil and cinnamon bark oil (see section 3.2).

It is a “top 100” substance and classified as R43 (IFRA, pers. comm.2010).

FARNESOL

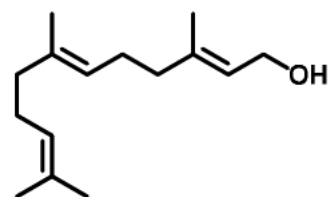
CAS # 4602-84-0

EC # 225-004-1

3,7,11-Trimethyl-2,6,10-Dodecatrien-1-ol

Farnesol; 3,7,11-Trimethyl-2,6,10-dodecen-1-ol; FCI 119a; Farnesyl alcohol; NSC 60597; Nikkosome

Current regulation: Annex III, part 1, n° 82



Clinical data:

In the “background information” section of the 1999 opinion, farnesol is classified as “less frequently reported allergen”; in 1 study of patients with cosmetic dermatitis 2 cases with contact allergy to farnesol had been reported; in other studies, positive reactions were seen in patients with positive PT reactions to MPR (33).

Since the last SCCNFP-opinion of 1999, farnesol is used not only for its scent, but also for its (slight) antimicrobial activity, useful, for instance, in deodorants. Thus, axillary dermatitis is a relatively typical presentation (111). In a multicentre study based on 1997/98 PT data, 0.5% positive reactions in consecutive patients were noted (Frosch 2002 a (16)). Farnesol is included in the FM II. In the original publication on single constituents of the FM II, 6 of 1701 consecutive patients reacted positively to farnesol 5%, ie., 0.35% (95% CI: 0.13 – 0.77%) (10). In a study on consecutive patients tested in 2003, 38 of 4238 patients had positive reactions to farnesol 5% pet. (0.9%, 95% CI: 0.6 – 1.2%) (4)(IVDK 2007). (A paper on farnesol previously published by the IVDK (112) presents results included in this later analysis.) In a series from Nagoya, Japan, 1.1% positive reactions in 1483 patients with suspected cosmetic dermatitis were observed (tested at 5% pet.) (14). In the Groningen 2009 study, 0.9% (95% CI: 0.2 – 2.7%) had positive reactions (6).

Additional information:

“Farnesol is an acyclic primary sesquiterpene alcohol found in essential oils such as lemongrass, citronella, tuberose blossom, sandalwood and orange blossom” (23). A RIFM review is available (113).

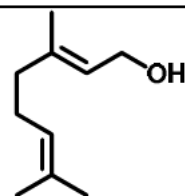
GERANIOL

CAS # 106-24-1

EC # 203-377-1

(2E)-3,7-Dimethyl-2,6-octadien-1-ol

(E)-3,7-Dimethyl-2,6-octadien-1-ol; (E)-Geraniol; (E)-Nerol; 3,7-Dimethyl-trans-2,6-octadien-1-ol; Geraniol; Geranyl alcohol; Lemonol; MosquitoSafe; NSC 9279; trans-3,7-Dimethyl-2,6-octadien-1-ol; trans-Geraniol; β-Geraniol



Current regulation: Annex III, part 1, n° 78

Clinical data:

In the “background information” section of the previous opinion (33), geraniol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 0.4% in consecutive PT patients and accounting for 3 to 7% of reactions to the FM I. Allergic reactions had been observed in 1.2 – 30% of patients with

eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 0.5% (95% CI: 0.2 – 0.9%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at 2%, i.e. twice the usual concentration (6). In a series from Nagoya, Japan, 0.3% positive reactions in 1483 patients with suspected cosmetic dermatitis were observed (tested at the unusually high concentration of 5% pet.) (14). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=7 (0.9%) positive reactions (22). The IVDK 2010 study, 0.39% (95% CI: 0.10 – 0.69%) of 1214 consecutively tested patients reacted to the compound, while 0.87% (95% CI: 0.63 – 1.10%) of 5695 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=17 reacted positively to geraniol (48).

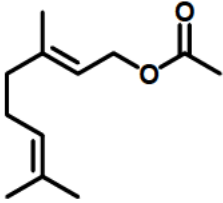
The fact that geraniol also occurs in food flavourings, and can elicit signs and symptoms of manifest contact sensitisation, is illustrated by the case of a 19 year old Japanese woman with cheilitis due to geraniol, improving after avoidance of respective foodstuff (114). A 20 year old Japanese woman with urticaria at the site of application of cosmetics with generalisation (contact urticaria syndrome grade 2), which A. Yamamoto et al. diagnosed as immediate type hypersensitivity to geraniol (without CA) (115).

Additional information:

Geraniol is a component of Palmarosa oil (CYMBOPOGON MARTINI see below), geranium oil (about 40%), citronella oil (30-40%), rose oil, lavender oil, and jasmine oil. It is sensitive to heat which induces autooxidation and isomeric with linalool (53).

Geraniol forms oxidation product with increased sensitizing capacity both via spontaneous autooxidation at air exposure and via metabolic oxidation. Geraniol and neral together with hydroperoxide have been identified as oxidation products when geraniol autooxidizes (84). Geraniol and neral were also identified as metabolites of geraniol (85). This explains the simultaneous reactions to geraniol and citral seen by (4).

A review is available by Hostynek and Maibach (116) and by RIFM (117). It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

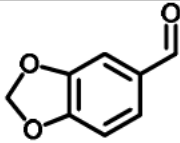
GERANYL ACETATE	
CAS # 105-87-3	
EC # 203-341-5	
(2E)-1-Acetate-3,7-dimethyl-2,6-octadien-1-ol	
(E)-Acetat-3,7-dimethyl-2,6-Octadien-1-ol; Geraniol acetate; (E)-3,7-Dimethyl-2,6-octadien-1-ol acetate; (E)-3,7-Dimethyl-2,6-octadienyl acetate; Acetic acid (2E)-3,7-dimethyl-2,6-octadienyl ester; Acetic acid geraniol ester; Bay pine (oyster) oil; Geranyl acetate; Geranyl ethanoate; NSC 2584; trans-1-Acetoxyl-3,7-dimethyl-2,6-octadiene; trans-3,7-Dimethyl-2,6-octadien-1-yl acetate; trans-Geranyl acetate; β-Geranyl acetate	

Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010).

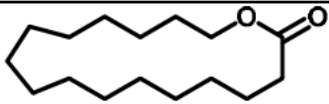
HELIOTROPINE	
CAS # 120-57-0	
EC # 204-409-7	
1,3-Benzodioxole-5-carboxaldehyde	
Piperonal; 2H-Benzo[3,4-d]-1,3-dioxolan-5-ylformaldehyde; 3,4-(Methylenedioxy)benzaldehyde; 3,4-Dihydroxybenzaldehyde methylene ketal; 3,4-Dimethylenedioxybenzaldehyde; 5-Formyl-1,3-benzodioxolane; 5-Formyl-1,3-benzodioxole; 5-Formylbenzodioxole; Benzo[1,3]dioxole-5-carbaldehyde; Benzo[d][1,3]dioxole-5-carboxaldehyde; Heliotropin; Heliotropine; NSC 26826; Piperonaldehyde; Piperonylaldehyde; Protocatechuic aldehyde methylene ether	

Current regulation: /

Clinical data:

In the Frosch 2002 b study, n=2 (0.2%) positive reactions to "piperonal" (1% pet.) and n=6 (0.4%) to "piperonal" (5% pet.), respectively, in 1606 consecutive were observed (17). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% heliotropine in pet., tested in 106 consecutive patients in Barcelona, were observed (15).

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

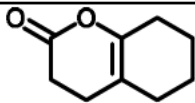
HEXADECANOLACTONE	
CAS # 109-29-5	
EC # 203-662-0	
Oxacycloheptadecan-2-one	
o-Lactone-16-hydroxy-hexadecanoic acid; 1,16-Hexadecanolide; 16-Hexadecanolactone; Cyclohexadecanolide; Dihydroambrettolide; Hexadecanoic acid, 16-Hydroxy-, o-lactone; Hexadecanolactone; Hexadecanolide; Juniperic acid lactone; NSC 33546	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 1 of 178 patients with previously diagnosed contact allergy to fragrance ingredients had a positive PT reaction to this compound, tested 5% pet. (19). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reactions to 5% "hexadecanolide" (13).

Additional information: /

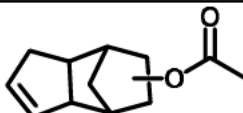
HEXAHYDROCOUMARIN	
CAS # 700-82-3	
EC # 211-851-4	
3,4,5,6,7,8-Hexahydro-2H-1-benzopyran-2-one	
3,4,5,6,7,8-Hexahydro-coumarin; δ -Lactone-2-hydroxy-1-cyclohexene-1-propanoic acid; 3,4,5,6,7,8-Hexahydrocoumarin; Hexahydrocoumarin; Δ -1,6-2-Oxabicyclo(4.4.0)decen-3-one	

Current regulation: Annex II, n° 1135

Clinical data: /

Additional information:

A RIFM review is available (93), p. S115 ff, citing a number of positive human sensitisation experiments.

3α,4,5,6,7,7α-HEXAHYDRO-4,7-METHANO-1H-INDEN-5(OR 6)-YL ACETATE	
CAS # 54830-99-8	
EC # 259-367-2	
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-indenol Acetate	
Acetoxidyhydrodicyclopentadiene; Cyclacet; Dicyclopentenyl acetate; Dicylat; Tricyclo[5.2.1.0 ^{2,6}]dec-3-enyl acetate; Tricyclodecenyyl acetate	

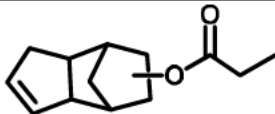
Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 1 to 5% "Cyclacet ®" in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15).

Additional information:

Produced by IFF under the brand name "Cyclacet" (<http://www.iff.com/Ingredients.nsf/0/1C9F2CB39EB1EF6480256993002FBC14>, last accessed 2010-07-08).

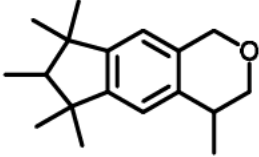
HEXAHYDRO-METHANOINDENYL PROPIONATE	
CAS # 68912-13-0	
EC # 272-805-7	
3α,4,5,6,7,7α-Hexahydro-4,7-methano-1H-indenol propanoate	

3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-indenyl propionate (Mixture of Isomers); Dicyclopentadiene propionate; tricyclodecenyl propionate; Tricyclo[5.2.1.0 ^{2,6}]dec-3-enyl propionate; Verdyl propionate	
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Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

HEXAMETHYLINDANOPYRAN	
CAS # 1222-05-5	
EC # 214-946-9	
1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[γ]-2-benzopyran	
1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[γ]-2-benzopyrane; 1,3,4,6,7,8-Hexahydro-4,6,6,8,8,8-hexamethylcyclopenta-2-benzopyran; Abbalide; Galaxolide; Galaxolide 50; Galaxolide 50BB; Galaxolide 50IPM; Galaxolide White; HHCB; Pearlide	

Current regulation: /

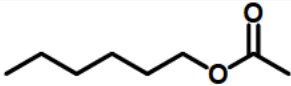
Clinical data:

In the Frosch 2002 a study, n=3 (0.2%) had positive reactions to the compound, tested 10% in isopropyl myristate (with 1 patient reacting positively to the diluent) (16). The Larsen 2001 study, testing with HHCB 7% pet., found 3.4% positive reactions in 178 patients with known contact allergy to fragrance ingredients (19). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had a positive reaction to "Galaxolide 50", tested at 5% (13) (test concentration 2% pet.). The DeGroot 1985 study identified 3 (1.7%) positive reactions among 179 patients using a 25% PT preparation of HHCB (25). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% "Galaxolide 50 ®" in pet., tested in 100 consecutive patients in Stockholm, were observed (15).

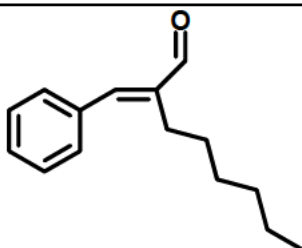
Additional information:

[0403/00 - Opinion concerning Hexahydro-hexamethyl-cyclopenta\(γ\)-2-benzopyran \(HHCB\)](#)

[0610/02 - Opinion on Hexahydro-hexamethyl-Cyclopenta \(γ\)-2-Benzopyran \(HHCB\)](#) (no restrictions) It is a "top 100" substance (IFRA, pers. comm.2010).

HEXYL ACETATE	
CAS # 142-92-7	
EC # 205-572-7	
Hexyl ethanoate	
Acetic acid, hexyl ester, Hexyl alcohol, acetate; 1-Hexyl acetate; Exceed 600; Hexyl acetate; Hexyl ester acetic	

acid;; NSC 7323; n-Hexyl acetate; n-Hexyl ethanoate	
Current regulation: /	
Clinical data: /	
Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).	

HEXYL CINNAMAL	
CAS # 101-86-0	
EC # 202-983-3	
α-Hexyl-cinnamaldehyde	
2-(Phenylmethylene)octanal; 2-Hexyl-3-phenyl-2-propenal; 2-Hexylcinnamaldehyde; Hexyl cinnamic aldehyde; NSC 406799; NSC 46150; α-Hexylcinnamaldehyde; α-Hexylcinnamic aldehyde; α-Hexylcinnamyl aldehyde; α-n-Hexyl-β-phenylacrolein; α-n-Hexylcinnamaldehyde	
Current regulation: Annex III, part 1, n° 87	

Clinical data:

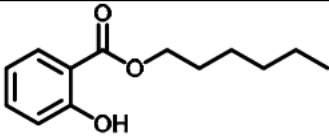
In the "background information" section of the 1999 opinion, hexyl cinnamal (synonymous: alpha-hexyl cinnamal, AHCA) is classified as "less frequently reported allergen"; 2 studies with 1 case and 1 study with 7 cases of contact allergy to this compound in patients with eczema from cosmetic products were found (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 0.3% positive PT reactions to consecutive patients were noted (16). In the subsequent EU 2005 study, 2 of 1701 patients had positive reactions to AHCA, and n=16 doubtful or irritant to AHCA at 10% in pet. (10). The IVDK 2007 study yielded n=3, i.e. 0.2% (95% CI: 0.03 – 0.4%) positive reactions in 2019 consecutively PTed patients, using 10% pet. as test concentration (4). In the Groningen 2009 study, 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, using a lower test concentration of 5% pet. (6).

Additional information:

It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

Hexyl cinnamal is regarded as "a recommended positive control for skin sensitization testing", e.g., in the context of the LLNA (118).

HEXYL SALICYLATE	
CAS # 6259-76-3	
EC # 228-408-6	
Hexyl-2-hydroxybenzoate	
Salicylic acid, hexyl ester; 1-Hexyl salicylate; Hexyl salicylate; n-Hexyl salicylate	

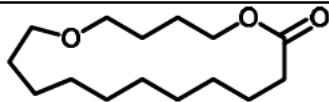
Current regulation: /

Clinical data:

None of the 218 patients with known contact allergy to fragrance ingredients reacted positively to this compound (tested at 5% in pet.) in the Larsen 2002 c study (1).

Additional information:

In a RIFM review, 2 human sensitisation experiments are mentioned which yielded no evidence of sensitising potential (HRIPT, n=103, maximisation test, n=22) (119). It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

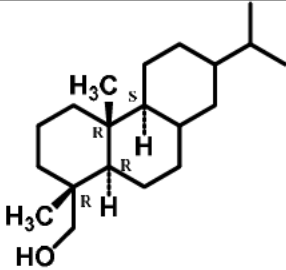
HIBISCOLIDE	
CAS # 6707-60-4	
EC # 229-755-6	
1,6-Dioxacycloheptadecan-7-one	
Undecanoic acid, 11-(4-hydroxybutoxy)-, o-lactone; 12-Oxa-1,16-hexadecanolide; Cervolide; Musk 781; NSC 34741; 12-Oxahexadecan-16-olide	

Current regulation: /

Clinical data:

None of the 178 patients with known contact allergy to fragrance ingredients reacted positively to "12-oxahexadecanolide" (tested at 5% in pet.) in the Larsen 2001 study (19).

Additional information: /

HYDROABIETYL ALCOHOL, when used as a fragrance ingredient	
CAS # 13393-93-6	
EC # 236-476-3	
(1R,4aR,4βS,10aR)-Tetradecahydro-1,4a-dimethyl-7-(1-methylethyl)-1-Phenanthrenemethanol	
Tetradecahydro-1,4a-dimethyl-7-(1-methylethyl)-phenanthrenemethanol; Tetrahydroabietyl alcohol	1-


Current regulation: AnnexII, n° 440

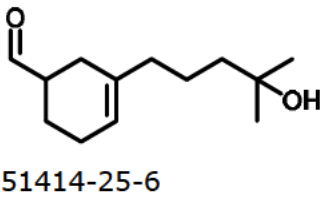
Clinical data:

In the deGroot 2000 study, 17 of 1825 consecutively tested patients had positive reactions to hydroabietyl alcohol (10% pet.) (12).

Additional information:

Commercial hydroabietyl alcohol consists of di- and tetrahydroabietyl alcohol together with non-modified colophony (120)

HYDROXYISOHEXYL	3-CYCLOHEXENE	
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CARBOXALDEHYDE (HICC) regioisomers	
CAS # 31906-04-4 / 51414-25-6	
EC # 250-863-4 / 257-187-9	
4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (31906-04-4) 3-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (51414-25-6)	

31906-04-4: 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexenecarboxaldehyde;
 4-(4-Methyl-4-hydroxyamyl)cyclohex-3-ene carboxaldehyde; Lyrall

Current regulation: Annex III, part 1, n° 79

Clinical data:

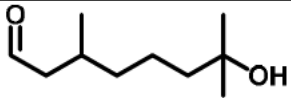
In the "background information" section of the previous opinion (33) HICC is classified as frequent allergen, causing allergic reactions in about 2.8% in consecutive PT patients, two thirds of these being relevant (33).

Since the last SCCNFP-opinion of 1999, in the Frosch 2002 a study, 2.7% of the 1855 consecutive patients reacted positively to HICC (5% pet.) (16). In the EU 2005 study, 28 of 1701 patients (1.7%, 95% CI: 1.1 – 2.4%) reacted positively to 5% HICC in pet. (10). In 21325 patients PTed consecutively in the IVDK 2007 study, 2.4% (95% CI: 2.2 – 2.6%) positive reactions were noted to 5% HICC in pet. (4). Similar to other studies, HICC was the most common single fragrance allergen among 320 patients tested in the Groningen 2009 study, with 3.1% (95% CI: 1.5 – 5.7%) positive reactions despite testing with a lower concentration of 2% pet. (6). In the An 2005 study, 7 of 422 consecutive patients, i.e., 1.7%, had positive reaction (13). The Belsito 2006 study (20) yielded a relatively low prevalence of 0.4% (7 of 1603; exact 95% CI (recalculated): 0.17 – 0.90%) positive reactions with 5% HICC in pet. and even less with lower test concentrations; possible reasons for the much lower prevalence were discussed. The IVDK 2010 study, 2.36% (95% CI: 2.19 – 2.53%) of 37270 consecutively tested patients reacted to HICC (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=8 reacted positively to HICC (48).

Further clinical data with a focus on quantitative dose-response (see also section 4.3), is discussed in (121).

Among the early case reports, S.A. Hendriks reported the case of a 20 year old patient developing axillary dermatitis after 5 months use of a deodorant containing HICC (122).

Additional information: /

HYDROXYCITRONELLAL	
CAS # 107-75-5	
EC # 203-518-7	
7-Hydroxy-3,7-dimethyl-octanal	

(±)-Hydroxycitronellal; 3,7-Dimethyl-7-hydroxyoctanal; 7-Hydroxy-3,7-dimethyloctanal; 7-Hydroxycitronellal; Citronellal hydrate; Citronellal, hydroxy-; Cyclalia; Cyclosia; Cyclosia base; Fixol; Hydroxycitronellal; Laurine; Lilyl aldehyde; Muguet synthetic; Muguetine principle; NSC

406740; Phixia

Current regulation: Annex III, part 1, n° 72

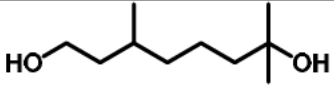
Clinical data:

In the “background information” section of the previous opinion (33), hydroxycitronellal, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 0.75% in consecutive PT patients and accounting for 6 to 16% of reactions to the FM I. Allergic reactions had been observed in 10 – 45% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.3% (95% CI: 0.9 – 1.9%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 2.2% (95% CI: 0.9 – 4.5%) had positive reactions to this compound, tested at 2% pet., i.e., twice the commonly used concentration (6). The Sugiura 2000 study observed 1% positive PT reactions (test concentration 5% pet.) in 1483 patients tested for suspected cosmetic dermatitis (14). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.5% positive reactions (22). The IVDK 2010 study, 1.17% (95% CI: 0.48 – 1.85%) of 1214 consecutively tested patients reacted to the compound, while 2.95% (95% CI: 2.43 – 3.47%) of 4359 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were tested with hydroxycitronellal, yielding 6 positive reactions (48).

Additional information:

Hydroxycitronellal is a synthetic fragrance, which only recently has been found in a few essential oils, e.g., of a *Narcissus* species and in essential oils of pepper (53)

HYDROXYCITRONELLOL	
CAS # 107-74-4	
EC # 203-517-1	
3,7-Dimethyl-7-octanediol	
2,6-Dimethyl-2,8-octanediol; 3,7-Dimethyl-1,7-octanediol; 3,7-Dimethyloctan-1,7-diol; Citronellol, hydroxy-; Hydroxyciol; Hydroxycitronellol; NSC 406140; NSC 67886	

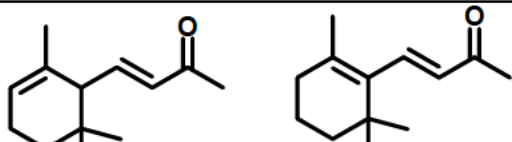
Current regulation: /

Clinical data:

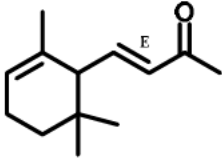
This compound elicited 6.0% positive PT reactions in 218 fragrance sensitive individuals (Larsen 2002 c, (1)).

Additional information:

A RIFM review is available, reporting results of a human induction study (maximisation test) in 25 volunteers, yielding no evidence of sensitisation (123).

IONONE isomeric mixture	
CAS # 8013-90-9	
EC # 232-396-8	

Ionone	
Irisone, mixture of alpha- and beta ionone	
Current regulation: /	
Clinical data: / (see single isomers)	
Additional information:	
It is a "top 100" substance, further specified with "mixed isomers" (IFRA, pers. comm.2010).	
INCI: "MIXED IONONES", with CAS # 14901-07-6 / 6901-97-9 / 8013-90-9 (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=35383 , last accessed 2010-07-13).	
A RIFM review is available on "ionone" (124), quoting negative human and experimental results.	

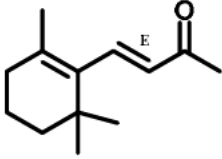
alpha-IONONE	
CAS # 127-41-3	
EC # 204-841-6	
(3E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one	
(E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-Buten-2-one; (5E)-Ionone; (E)-4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; (E)-α-Ionone; (±)-trans-α-Ionone; (±)-α-Ionone; 4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; 4-(2,6,6-Trimethyl-2-cyclohexenyl)-3-buten-2-one; trans-α-Ionone; α-Cyclocitrylideneacetone; α-Ionone	

Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% alpha-ionone in pet., tested in 205 consecutive patients, were observed (15).

Additional information: A RIFM review is available (125).

beta-IONONE	
CAS # 79-77-6	
EC # 201-224-3	
(3E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	
(E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one; (E)-β-Ionone; Ionone beta; trans-β-Ionone; β-Ionone	

Current regulation: /

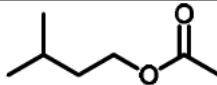
Clinical data:

In the Frosch 1995 dose finding pilot study, no positive reaction to 1% and 5% beta-

ionone in pet., tested in 205 consecutive patients, were observed (15).

Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010). A RIFM review is available (126).

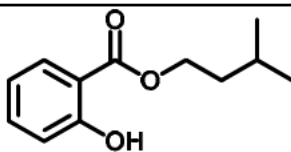
ISOAMYL ACETATE	
CAS # 123-92-2	
EC # 204-662-3	
3-Methylbutyl acetate	
1-Butanol, 3-methyl-, acetate; Acetic acid, isoamyl ester; Isopentyl alcohol, acetate; 3-Methyl-1-butanol acetate; 3-Methyl-1-butyl acetate; 3-Methylbutyl acetate; 3-Methylbutyl ethanoate; Acetic acid 3-methyl-1-butyl ester; Acetic acid 3-methylbutyl ester; Acetic acid isopentyl ester; Banana oil; Isoamyl acetate; Isoamyl alcohol acetate; Isoamyl ethanoate; Isopentyl acetate; Isopentyl ethanoate; NSC 9260; Pear oil; i-Amyl acetate; iso-Amyl acetate; iso-Pentyl acetate	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

In CosIng, it is listed as "solvent"
<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=76810>,
 last accessed 2010-07-13)

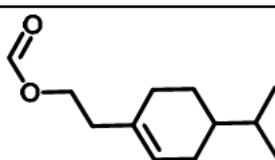
ISOAMYL SALICYLATE	
CAS # 87-20-7	
EC # 201-730-4	
3-Methylbutyl-2hydroxybenzoate	
Isopentyl 2-Hydroxybenzoate; Isopentyl salicylate; Salicylic acid, isopentyl ester (6CI,8CI); Isopentyl alcohol, salicylate; 3-Methylbutyl salicylate; Isoamyl o-hydroxybenzoate; Isoamyl salicylate; Isopentyl salicylate; NSC 7952	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified 1 (0.6%) positive reactions among 179 patients using a 50% PT preparation of this compound – this reaction may have been due to an “excited back syndrome” and is thus a limited evidence (25). In the Frosch 1995 dose finding pilot study, no positive reaction to 1% and 5% isoamyl salicylate in pet., tested in 95 consecutive patients, were observed (15).

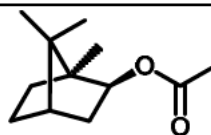
Additional information: A RIFM review is available (127).

ISOBERGAMATE	
CAS # 68683-20-5	
EC # 272-066-0	
4-(Isopropyl)cyclohexadiene-1-ethyl formate	
Structure is incompletely defined 4-(1-Methylethyl)-1,?-cyclohexadiene-1-ethyl formate 4-(Isopropyl)cyclohexadiene-1-ethyl methanoate; menthadienyl formate; Menthadiene-7-methyl formate	

Current regulation: Annex III, part 1, n° 170

Clinical data: /

Additional information: A RIFM review is available (128).

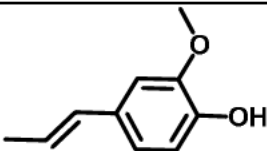
ISOBORNYL ACETATE	
CAS # 125-12-2	
EC # 204-727-6	
(1R,2R,4R)-1,7,7-trimethyl-Bicyclo[2.2.1]hept-2-yl acetate	
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-Trimethyl-, acetate, (1R,2R,4R)-rel- ; Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, exo-; Isoborneol, acetate; (±)-Isobornyl acetate; Isobornyl acetate; NSC 62486; Pichtosin; Pichtosine; exo-Bornyl acetate	

Current regulation: /

Clinical data:

In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% isobornyl acetate in pet., tested in 107 consecutive patients in High Wycombe, were observed (15).

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

ISOEUGENOL	
CAS # 97-54-1	
EC # 202-590-7	
2-Methoxy-4-(1-propen-1-yl)-phenol	
Phenol, 2-methoxy-4-(1-propenyl)- ; Phenol, 2-methoxy-4-propenyl-; 1-(3-Methoxy-4-hydroxyphenyl)-1-propene; 2-Methoxy-4-(1-propenyl)phenol; 2-Methoxy-4-propenylphenol; 3-Methoxy-4-hydroxy-1-propenylbenzene; 4-Hydroxy-3-methoxy-1-propenylbenzene; 4-Hydroxy-3-methoxy-β-methylstyrene; 4-Propenylguaiacol; Isoeugenol; NSC 6769	

Current regulation: Annex III, part 1, n° 73

Clinical data:

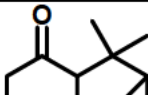
In the “background information” section of the previous opinion (33), isoeugenol, one of the 8 components of the FM I, is classified as frequent allergen, causing allergic reactions in about 1.9% in consecutive PT patients and accounting for 6 to 22% of reactions to the FM I. Allergic reactions had been observed in 2 – 25% of patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, the IVDK 2007 study yielded 1.3% (95% CI: 0.8 – 1.8%) positive reactions in 2063 consecutively PTed patients (4). In the Groningen 2009 study, 1.3% (95% CI: 0.3 – 3.2%) had positive reactions to isoeugenol, tested at 2% pet., i.e., twice the commonly used concentration (6). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 5.4% positive reactions (22). At St Johns Institute of Dermatology in London 3636 subjects were patch tested with isoeugenol 2001-2005, 97 of whom were positive. Year-on-year incidence showed an increasing trend, with an overall incidence of 2.67% (129). The IVDK 2010 study, 1.62% (95% CI: 0.87 – 2.38%) of 1214 consecutively tested patients reacted to the compound, while 3.41% (95% CI: 2.90 – 3.92%) of 5747 of patients tested in a more aimed manner, partly as break-down testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=11 reacted positively to isoeugenol (48).

Additional information:

Isoeugenol occurs in a cis- (CAS 5912-86-7) and a trans-isomers (CAS 5932-68-3), the latter dominating in trade products (82-88%) (53).

Isoeugenyl methyl ether caused 7.3% positive reactions in the Larsen 2002 c study (1). A number of derivatives of isoeugenol, such as isoeugenyl acetate, transisoeugenol, isoeugenyl benzoate, isoeugenyl phenylacetate, isoeugenyl methyl ether and benzyl isoeugenyl have been examined in 2261 consecutive patients; a varying proportion of positive patch test reactions and a varying proportion of concomitant reactions with isoeugenol have been observed (130). In an earlier study, 5 of 7 patients positive to isoeugenol also displayed positive reactions to isoeugenol acetate (1.2% eth.) (131) (see also section 5 and 6).

SOLONGIFOLENEKETONE	
CAS # 33407-62-4	

EC # 245-890-3	
1,3,4,6,7,8a-Hexahydro-1,1,5,5-tetramethyl-2H-2,4a-methanonaphthalen-8(5H)-one	
Hexahydro-1,1,5,5-tetramethyl-2H-2,4a-methanonaphthalen-8(5H)-one	

Current regulation: /

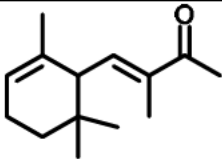
Clinical data:

The Larsen 2001 study identified 1 in 178 patients with known contact allergy to fragrance ingredients who reacted positively in the PT (5% pet.) (19).

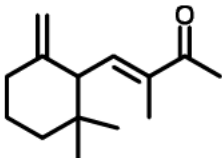
Additional information:

Not listed in CosIng under this CAS #. Other CAS # reported in RIFM review ¹³:

- 29461-14-1 CosIng: INCI name "ISOLONGIFOLENE KETONE EXO";
- 23787-90-8 CosIng: INCI name "ISOLONGIFOLANONE";
- 29461-13-0: CosIng: INCI name "HEXAHYDRO-TETRAMETHYLMETHANONAPHTHALEN-8-ONE".

<i>alpha-ISOMETHYL IONONE</i>	
CAS 127-51-5	
EC 204-846-3	
3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	
4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-methyl-3-buten-2-one; Cetone Alpha; Isomethyl-α-ionone; NSC 66432; α-Cetone	

Current regulation: Annex III, part 1, n° 90

<i>gamma-Methylionone</i>	
CAS 7388-22-9	
EC /	

According to CosIng, "alpha-ISOMETHYL IONONE" (CAS # 127-51-5) and "gamma-Methylionone" (CAS # 7388-22-99) are synonyms, with one CAS number, and one preferred chemical name. The substance(s) are accordingly treated in the 1999 opinion (33) as one. As this treatment is also found in the literature, both substances are reviewed together.

¹³ Opdyke, D. L. J.; Letizia, C. **Monographs on fragrance raw materials. Isolongifolanone.** Food and Chemical Toxicology (1983), 21(6), 859

Clinical data:

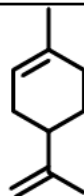
In the “background information” section of the 1999 opinion, “gamma-methylionone” is classified as “less frequently reported allergen”; 1 study with 2 cases and 2 studies with 1 case were found among patients with eczema from cosmetic products (33).

The IVDK 2007 study yielded $n=1$, i.e. 0.1% (95% CI: 0.00 – 0.2%) positive reactions in 2004 consecutively PTed patients (4). In the subsequent period (2005-2008), $n=986$ patients were tested in the IVDK 2010 study, with no positive reactions (7). In the Groningen 2009 study, $n=2$, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen, tested at only 1% pet. (6). In a Korean study with 422 consecutive patients, 2.1% reacted positively to “alpha isomethyl ionone (gamma-methylionone), CAS # 127-51-5”, tested 5% pet. (13)

Additional information:

It is a “top 100” substance (IFRA, pers. comm.2010) under the label of “alpha-ISOMETHYL IONONE (CAS # 127-51-5)”.

A RIFM review is available, listing 4 human sensitisation experiments employing different study protocols – all yielding negative results (132). Another review is available by Hostynek and Maibach (133), both referring to “alpha-ISOMETHYL IONONE (CAS # 127-51-5)”.

(DL)-LIMONENE	
CAS # 138-86-3	
EC # 231-732-0	
1-Methyl-4-(1-methylethenyl)-cyclohexene	
p-Mentha-1,8-diene; (±)-Dipentene; (±)-Limonene; (±)-α-Limonene; 1,8-p-Menthadiene; 1-Methyl-4-(1-methylethenyl)cyclohexene; 1-Methyl-4-isopropenyl-1-cyclohexene; 1-Methyl-4-isopropenylcyclohexene; 1-Methyl-p-isopropenyl-1-cyclohexene; 4-Isopropenyl-1-methyl-1-cyclohexene; 4-Isopropenyl-1-methylcyclohexene; Cajeputen; Cajeputene; Cinen; Cinene; DL-Limonene; Dipenten; Dipentene; Eulimen; Flavor orange; Goldflush II; Kautschin; Limonen; Limonene; NSC 21446; NSC 844; Nesol; Orange X; Orange flavor; PC 560; Roti-Histol; SF 001; dl-Limonene; α-Limonene	
Current regulation: Annex III, part1, n° 88, 167, 168	

Clinical data:

In the “background information” section of the 1999 opinion, d-limonene (CAS 5989-27-5) is classified as “less frequently reported allergen in relation to cosmetic exposure”; with contact allergy to oxidised limonene not infrequently reported in the literature (33).

Since 1999, several studies have been performed using limonene where the oxidation state is not given, but intended to be low. In one study, 0.6% positive reactions to limonene (3% pet.) were observed in 1606 consecutive patients (17). The IVDK 2007 study yielded n=3, i.e. 0.1% (95% CI: 0.03 – 0.4%) positive reactions in 2396 patients consecutively PTed with limonene (2% pet.) (4). The IVDK 2010 study, 0.28% (95% CI: 0 – 0.57%; percentages standardised for age and sex) of 1241 patients PTed with dipentene reacted to the compound (7). In the Groningen 2009 study, no positive reactions to this allergen, tested at 2% pet., were observed in 320 patients (6).

Regarding selected case reports, a case of a 40 year old citrus fruit picker with work related hand dermatitis and bronchial asthma has been described, who tested extreme positive to DL-limonene (2% pet.), and, less extremely, to citronellol and to the biocide dichlorophene (134). Moreover, limonene is used as a solvent in technical applications and cleaning and can lead to allergic contact dermatitis (e.g., a histopathology technicians (135, 136) or a painter and decorator (137)). In “water-free” hand cleansers it is reported to be used in concentrations around 10 – 20% (137). Wax polishes may contain dipentene and have caused one reported case of occupational ACD in a car mechanic (138). Another case of occupational ACD from dipentene in honing oil has been reported (139). In a case series from Sweden, 2 of 105 car mechanics patch tested for occupational contact dermatitis had positive reactions to oxidised d-limonene (5% pet.) (140).

Additional information:

Limonene is a monocyclic monoterpene existing in two enantiomers: (R)-(+)-limonene (CAS 5989-27-5) and (S)-(-)-limonene (CAS 5989-54-8). Racemic limonene is known as dipentene.

The allergenicity of limonene is closely related to oxidation (71, 72, 141, 142). It has been demonstrated that both enantiomers, R-(+)- and S-(-)-limonene spontaneously autoxidize, and that the primary oxidation products formed, the hydroperoxides, are strong and clinically relevant contact allergens. Among 2411 consecutive patients in a multi-centre European study, 63 (2.6 % [95%CI: 2.0-3.3]) reacted to oxidised (R)-(+)-and/or (S)-(-)-limonene (3.0% pet.) (72). In other multi-studies also, a considerable proportion of patients showed

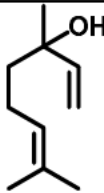
positive patch test reactions to oxidised R-(+)- limonene, e.g.,

- between 0.3% and 5.1% of subgroups of 2800 patients in Stockholm and Leuven, depending on test concentration, oxidation state and department(141),
- between 0.3% and 6.5% in 4 different departments in altogether 2273 patients (72, 143).

The primary oxidation products are the major allergens forming specific antigens (Bråred-Christensson J, Matura M, Bäcktorp C, Börje A, Nilsson JLG, Karlberg A-T. Hydroperoxides form specific antigens in contact allergy. Contact Dermatitis 2006; 55: 230-237.).

Current IFRA standards emphasise "a peroxide value of less than 20 millimoles peroxides per litre, determined according to the FMA method" (<http://www.ifraorg.org/Home/Code,+Standards+Compliance/IFRA+Standards/page.aspx/56>, last accessed 2009-11-11). For a more general discussion see section 5.

There is no scientific rationale for the difference in peroxide value allowed for limonene (20 millimoles peroxides per litre) compared to linalool (10 millimoles peroxides per litre). Specific values for hydroperoxides, which are allergens, would be desirable.

LINALOOL	
CAS # 78-70-6 (isomeric mixture)	
EC # 201-134-4; 245-083-6	
See: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=27933	
3,7-Dimethyl-1,6-octadien-3-ol	
(±)-Linalool; 2,6-Dimethyl-2,7-octadien-6-ol; 2-Methyl-1-prenyl-3-buten-2-ol; 3,7-Dimethyl-1,6-octadiene-3-ol; 3,7-Dimethyl-3-hydroxy-1,6-octadiene; L 260-2; Linalol; Linalool; Linalyl alcohol; Linanol; NSC 3789; dl-Linalool; β-Linalool	

Current regulation: Annex III, part 1, n° 84

Clinical data:
In the "background information" section of the 1999 opinion, linalool in non-oxidized form is classified as "less frequently reported allergen"; with 4 cases of contact allergy reported in 2 studies on patients with eczema from cosmetic products (33).

Since the last SCCNFP-opinion of 1999, studies have been performed on contact allergy to linalool, oxidation state not given, but intended to be low. In the Larsen 2002 c study, none of the 218 patients with known contact allergy to fragrance ingredients had a positive reaction to linalool 5% pet., as prepared specially for this study (1). The IVDK 2007 study yielded 0.3% (95% CI: 0.1 – 0.6%) positive reactions in 2401 patients consecutively tested with stabilised linalool (10% pet.) (4). The IVDK 2010 study, 1 patient had a weak, and another a ++ reaction among the n=985 patients tested with 10% linalool (stabilised) in pet. (7). In the Groningen 2009 study, n=2, i.e. 0.6% (95% CI: 0.1 – 2.2%) had positive reactions to this allergen (6). The deGroot 2000 study with 1825 consecutively tested patients yielded 3 positive reactions to linalool (12). The DeGroot 1985 study found no positive reactions among 179 patients using a 30 % PT preparation of linalool (25).

Additional information:

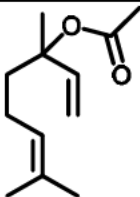
The allergenicity of linalool is closely related to oxidation and the primary oxidation products, the hydroperoxides, are the main allergens (144). In a clinical study 2002-

2003 in 6 European centres including 1511 consecutive patients, 1.3% showed a positive reaction to oxidized linalool (2.0% pet.) and 1.1% to the hydroperoxide fraction (65). A recent dose-response study in Sweden including 3400 patients in two test centres showed a positive reaction in 5.3% of the 1725 patients tested with oxidized linalool 6% pet. (145).

A review by RIFM is available both regarding linalool (146) and linalool "and related esters" (147). Another review is available by Hostynek and Maibach (148).

It is a "top 100" substance (IFRA, pers. comm.2010).

Additional CAS numbers exist for the single isomers: CAS # 126-90-9 (S-isomer), CAS # 126-91-0 (R-isomer); however, in the studies reviewed the isomeric mixture has been used throughout.

LINALYL ACETATE	
CAS # 115-95-7	
EC # 204-116-4	
3,7-Dimethyl-1,6-octadien-3-yl acetat	
1,6-Octadien-3-ol, 3,7-dimethyl-, acetate; Linalool acetate K; (±)-Linaloyl acetate; (±)-Linalyl acetate; 1,5-Dimethyl-1-vinyl-4-hexenyl acetate; 3,7-Dimethyl-1,6-octadien-3-yl acetate; 3-Acetoxy-3,7-dimethyl-1,6-octadiene; Acetic acid linalool ester; Bergamiol; Bergamol; Bergamot mint oil; Linalyl acetate; NSC 2138; dl-Linalool acetate	

Current regulation: /

Clinical data:
In 100 patients tested in Odense, DK, in the early 90s, no positive reactions were observed with 1 and 5% linalyl acetate in pet. (15). In the Frosch 2002 a study, testing with linalyl acetate (10% pet.), 0.2% positive PT reactions to consecutive patients were noted (16). Similarly, the RIFM review mentioned quotes a number of studies where no allergic reactions to this compound had been observed, with the exception of one positive reaction in a Dutch study in 1988(149).

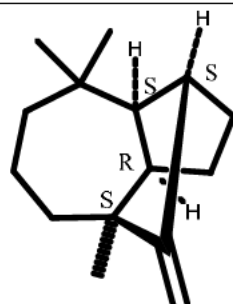
Additional information:

This is the main component of lavender oil (30%), also part of bergamot oil, neroli oil, peppermint oil, lemon oil and jasmine oil (53).

Linalyl acetate autoxidizes spontaneously at air exposure and the major allergens, the hydroperoxides, are the primary oxidation products (150). The pattern of autoxidation is similar to that for linalool and as the acetate can be metabolically hydrolysed to the corresponding alcohol cross reactions to allergens from oxidized linalool should be possible. This was indicated in a study of lavender oil and oxidised linalyl acetate which elicited positive PT reactions in some patients with known contact allergy to oxidised linalool (n=3) (151).

A RIFM review is available reporting 7 human sensitisation experiments yielding few or no cases of sensitisation (152).

It is a "top 100" substance (IFRA, pers. comm.2010).

Longifolene	
CAS # 475-20-7	
EC # 207-491-2	
(1S,3aR,4S,8aS)-Decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene	
1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, (1S,3aR,4S,8aS)-(+)-; 1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1a,3aβ,4a,8aβ)]-; (+)-Longifolene; Junipen; Junipene; Kuromatsuen; Kuromatsuene; Longifolen; NSC 150808; d-Longifolene; α-Longifolene	

Current regulation: /

Clinical data: /

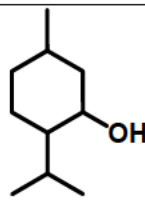
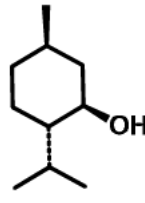
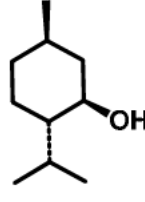
Additional information:

It is a "top 200" substance and classified as R43 (IFRA, pers. comm.2010)

http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=77412

This substance is listed in the Register of Flavouring Substances pursuant to Article 3(1) of Regulation EC No. 2232/96 (28 Oct 1996) that lays down a procedure for flavouring substances used or intended for use in or on foodstuffs. Adopted February 23, 1999.

A RIFM review is available citing one negative human maximisation test (n=25) with 10% pet. (153).

MENTHOL	 1490-04-6
CAS # 1490-04-6 / 89-78-1 / 2216-51-5	
EC # 216-074-4 / 239-388-3 / 218-690-9	
5-Methyl-2-(1-methylethyl)-cyclohexanol (1490-04-6) (1R,2S,5R)-rel-5-Methyl-2-(1-methylethyl)-cyclohexanol (89-78-1) (1R,2S,5R)-5-Methyl-2-(1-methylethyl)-cyclohexanol (2216-51-5)	
Other names:	 89-78-1
1490-04-6: Menthol; 1-Methyl-4-isopropyl-3-cyclohexanol; 2-Isopropyl-5-methylcyclohexan-1-ol; 2-Isopropyl-5-methylcyclohexanol; 3-Hydroxy-p-menthane; 5-Methyl-2-(1-methylethyl)cyclohexanol; 5-Methyl-2-isopropylcyclohexanol; Menthyl alcohol; p-Menthan-3-ol 89-78-1: (1a,2β,5a)-5-Methyl-2-(1-methylethyl)-cyclohexanol; cis-1,3,trans-1,4-Menthol; dl-Menthol; (1R,2S,5R)-rel-5-Methyl-2-(1-methylethyl)cyclohexanol; (±)-Menthol; DL-Menthol; Fisherman's Friend Lozenges; Hexahydrothymol; Menthacamphor; Menthol; Menthomenthol; NSC 2603; Peppermint camphor; Racementhol; Therapeutic Mineral Ice; Thymomenthol; rac-Menthol	
	 2216-51-5

2216-51-5: (1R,2S,5R)-5-Methyl-2-(1-methylethyl)-cyclohexanol; [1R-(1 α ,2 β ,5 α)]-5-Methyl-2-(1-methylethyl)-cyclohexanol; (1R,3R,4S)-(-)-Menthol; (-)-Menthol; (-)-Menthyl alcohol; (-)-trans-p-Methan-cis-3-ol; (1R)-(-)-Menthol; (1R,2S,5R)-(-)-Menthol; (1R,2S,5R)-2-Isopropyl-5-methylcyclohexan-1-ol; (1R,2S,5R)-2-Isopropyl-5-methylcyclohexanol; (R)-(-)-Menthol; 1R-Menthol; L-Menthol; L-Mentholum; Levomenthol; NSC 62788; l-(-)-Menthol; l-Menthol

Current regulation: /

Clinical data:

Among 512 patients referred from a dental department for diagnostic work-up of various intraoral symptoms and complaints within 4 years, 10 patients had positive (+ to +++) PT reactions to menthol 5% pet. at D4, mostly reporting dramatic improvement after cessation of use of peppermint-containing oral products (154). In 63 patients positive to the FM I, 1 had a positive PT reaction to menthol, 5% pet., in the Santucci 1987 study (28). The IVDK 2010 study, 1 of 1147 patients tested with 1% menthol in pet. had a weak positive reaction to menthol (7).

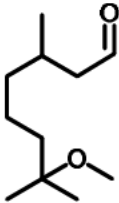
A case of contact allergy to "peppermint and menthol" in a transdermal therapeutic system with flurbiprofen for lumbar pain has been described (155). Moreover, a case of rhinitis caused by different menthol-containing products, diagnostically proven by repeatedly positive urticarial reactions after application of 2% menthol in pet. or 5% peppermint oil in pet., has been reported (156). "A case of asthma due to menthol is reported in a 40-year-old woman with no history of asthma or any other allergy. During the last two years, the patient had presented dyspnoea, wheezing and nasal symptoms when exposed to mentholated products such as toothpaste and candies. The aetiology was suggested by the history of exposure and diagnosis was established by skin tests and bronchial challenge with menthol. The patient achieved control of symptoms by avoiding menthol and its derivatives." (157).

Additional information:

Menthol is an ingredient of several essential oils, like peppermint oil, and has been identified as causative allergen in case reports listed above.

Four stereoisomeric forms are known. Natural menthol occurs as L-form (CAS 2216-51-5), trade products are DL-menthol (CAS 1490-04-6). D-form: CAS 89-78-1, racemic: CAS 15356-70-4. Sensitive to light, air and heat (53).

L-menthol and menthol (isomer not specified) are "top 100" substances (IFRA, pers. comm.2010). RIFM reviews are available regarding "menthol" (158), D-menthol (159), L-menthol (160), DL-menthol (161) and menthol, racemic (162). A CIR expert panel review is available (163).

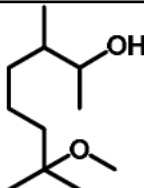
METHOXYCITRONELLAL	
CAS # 3613-30-7	
EC # 222-784-5	
7-Methoxy-3,7-dimethyl-octanal	
7-Methoxy-3,7-dimethyloctanal; 7-Methoxy-6,7-dihydrocitronellal; 7-Methoxycitronellal; Methoxycitronellal; Methoxydihydrocitronellal	

Current regulation: /

Clinical data:

Nakayama et al. found 1974 (after (29)) 12 "strong positive" and 10 "weak positive" reactions to methoxycitronellal (unknown test concentration), with cross-reactions to hydroxycitronellal (proportion not given), in 183 patients.

Additional information: /

METHOXYTRIMETHYLHEPTANOL	
CAS # 41890-92-0	
EC # 255-574-7	
7-Methoxy-3,7-dimethyl-2-octanol	
3,7-Dimethyl-7-methoxy-2-octanol; Dihydromethoxyelgenol; Elesant; Osyrol	

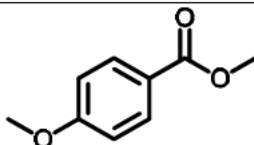
Current regulation: /

Clinical data:

In the Larsen 2002 c study, 0.9% of the patients with known contact allergy to fragrance ingredients had a positive PT reaction to this ingredient not reported as allergen previously (1).

Additional information:

A RIFM review is available (128) citing 1 negative maximisation test (n=27).

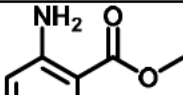
METHYL <i>p</i>-ANISATE	
CAS # 121-98-2	
EC # 204-513-2	
Methyl-4-methoxybenzoate	
p-Anisic acid, methyl ester; 4-(Methoxycarbonyl)anisole; 4-Methoxybenzoic acid methyl ester; Methyl p-anisate; Methyl p-methoxybenzoate; NSC 7324; p-Methoxybenzoic acid methyl ester	

Current regulation: /

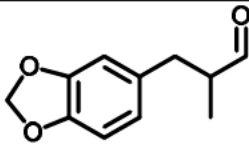
Clinical data:

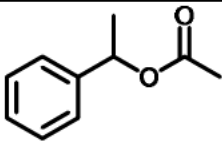
In the Malten 1984 study, n=1 (0.5%) of 182 patients displayed a positive PT reaction to methyl anisate 4% pet. (24).

Additional information: /

METHYL ANTHRANILATE	
CAS # 134-20-3	

EC # 205-132-4	
Methyl 2-aminobenzoate	
Anthranilic acid, methyl ester; 2-(Methoxycarbonyl)aniline; 2-Aminobenzoic acid methyl ester; 2-Carbomethoxyaniline; Bird Shield; Grain 96-1; Methyl 2-aminobenzoate; Methyl 6-aminobenzoate; Methyl anthranilate; Methyl o-aminobenzoate; NSC 3109; ReJex-iT; ReJex-iT AP 50; ReJex-iT TP 40; Sunarome UVA; [2-(Methoxycarbonyl)phenyl]amine; o-(Methoxycarbonyl)aniline; o-Aminobenzoic acid methyl ester; o-Carbomethoxyaniline	
Current regulation: /	
Clinical data:	
In 91 Israeli patients with a positive or doubtful reaction to FMI or MP methyl anthranilate was tested (conc. not given), with a negative result (164).	
Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).	

METHYLENEDIOXYPHENYL METHYLPROPANAL	
CAS # 1205-17-0	
EC # 214-881-6	
3-(1,3-Benzodioxol-5-yl)-2-methylpropanal	
Hydrocinnamaldehyde, α-methyl-3,4-(methylenedioxy)-; 2-Methyl-3-(3,4-methylenedioxyphenyl)propanal; 2-Methyl-3-(3,4-methylenedioxyphenyl)propionaldehyde; 3-(3,4-Methylenedioxyphenyl)-2-methylpropanal; Heliobouquet; Heliofresh; Heliogan; Helional; Helipropanal; NSC 22282; Tropional; α-Methyl-1,3-benzodioxole-5-propanal; α-Methyl-3,4-(methylenedioxy)hydrocinnamaldehyde	
Current regulation: /	
Clinical data: /	
Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).	

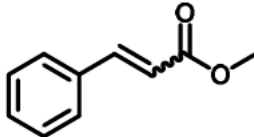
METHYLBENZYL ACETATE	
CAS # 93-92-5	
EC # 202-288-5	
1-Phenylethyl acetate	
Benzenemethanol, α-methyl-, acetate ; Benzyl alcohol, α-methyl-, acetate ; (±)-Styrallyl acetate; (±)-α-Methylbenzyl acetate; (±)-α-Phenethyl acetate; 1-Acetoxy-1-phenylethane; 1-Phenylethyl acetate; Gardeniol II; Gardenol; Methyl phenyl carbinyl acetate; Methylphenylcarbinol acetate; NSC 2397; Styrallyl acetate;	

Styrylallyl acetate; dl-1-Phenylethyl acetate; sec-Phenethyl acetate; sec-Phenylethyl acetate; α-Methylbenzenemethanol acetate; α-Methylbenzyl acetate; α-Methylbenzyl alcohol, acetate; α-Phenethyl acetate; α-Phenylethyl acetate

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

METHYL CINNAMATE	
CAS # 103-26-4	
EC # 203-093-8	
Methyl 3-phenylprop-2-enoate	
3-Phenyl-2-propenoic acid methyl ester; Cinnamic acid, methyl ester; 3-Phenyl-2-propenoic acid methyl ester; 3-Phenylacrylic acid methyl ester; Methyl 3-phenyl-2-propenoate; Methyl 3-phenylacrylate; Methyl 3-phenylpropenoate; Methyl cinnamate; Methyl cinnamylate; NSC 9411; SemaSORB 9815	

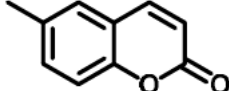
Current regulation: /

Clinical data:

Patch tests with some components of Peru balsam were carried out at 8 worldwide centers in 142 patients who had previously reacted to 25% MP. Reactions to methyl cinnamate (dose and vehicle not reported) were observed in 6 of 142 patients (no further details reported) (165).

Additional information:

A RIFM review is available (166), reviewing, e.g., a number of animal studies with conflicting results. See also under Myroxylon pereirae.

6-METHYL COUMARIN	
CAS # 92-48-8	
EC # 202-158-8	
6-Methylchromen-2-one	
Coumarin, 6-methyl-; 6-MC; 6-Methyl-2H-1-benzopyran-2-one; 6-Methyl-2H-chromen-2-one; 6-Methylbenzopyrone; 6-Methylcoumarin; 6-Methylcoumarinic anhydride; NSC 5870; Toncarine	

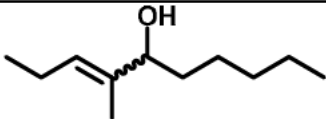
Current regulation: Annex III, part 1, n° 46

Clinical data:

Two of 24 white volunteers developed a photoallergic reaction after single epicutaneous exposure with 5% methyl coumarin in ethanol and UV-A radiation (16 J/cm²). After a photomaximisation test, 6 of 10 subjects developed photocontact allergic reactions

(167). Cardoso et al. report on 2 photoallergic patch test reactions to this substance, which were apparently clinically relevant, in 83 Portuguese patients tested (168). Similar results (2 of 76 patients with positive photopatchtest) were reported from New York (169).

Additional information: /

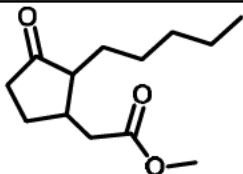
METHYL DECENOL	
CAS # 81782-77-6	
EC # 279-815-0	
4-Methyl-3-decen-5-ol	

Current regulation: /

Clinical data: /

Additional information:

A RIFM review is available (170), reporting 1 negative HRIPT (n=50). It is a "top 100" substance (IFRA, pers. comm.2010).

METHYL DIHYDROJASMONATE	
CAS # 24851-98-7	
EC # 246-495-9	
Methyl 2-(3-oxo-2-pentylcyclopentyl) acetate	

Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester; Kharismal; MDJ; Methyl (3-oxo-2-pentylcyclopentyl)acetate; Methyl 3-oxo-2-pentylcyclopentane ethanoate; Hedione

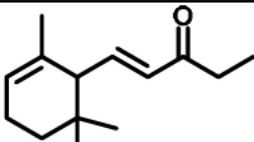
Current regulation: /

Clinical data:

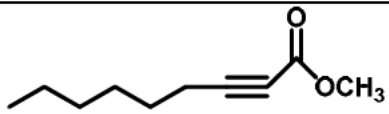
In the Frosch 2002 b study, 3 of 1606 consecutive patients (0.2%) showed positive reactions to hedione (5% pet.) (17). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% hedione in pet., tested in 100 consecutive patients in Belfast, were observed (15).

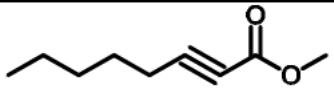
Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010). An older RIFM review exists (128) citing 1 negative human maximisation test (n=25).

METHYL IONONE (mixture of isomers)	
CAS # 1335-46-2	
EC # 215-635-0	

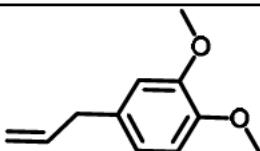
1-(2,6,6-Trimethyl-1-cyclohex-2-enyl)pent-1-en-3-one	
6-Methylionone	
Current regulation: /	
Clinical data:	
See METHYLIONANTHEME for one clinical case report. Regarding methyl ionone gamma, the Frosch 1995 dose-finding pilot study found no positive reaction to 1% and 5% of this substance in pet., tested in 100 consecutive patients in Belfast (15).	
Additional information:	
It is a "top 100" substance (IFRA, pers. comm.2010). A RIFM review is available (171).	

METHYL OCTINE CARBONATE	
CAS # 111-80-8	
EC #	
Methyl 2-octynoate	
Methyl 2-Nonynoate, MOC	
Current regulation: Annex III, part 1, n°173	
Clinical data:	
English and Rycroft reported a case of a 19-year-old laboratory technician working in the fragrance industry, who developed hand dermatitis after contact with methyl heptine and methyl octane carbonates; patch testing was strongly positive to both compounds at 1% in MEK (172).	
Additional information: /	

METHYL 2-OCTYNOATE	
CAS # 111-12-6	
EC # 203-836-6	
Methyl oct-2-ynoate	
M2O; Methyl heptin carbonate; Folione; Methyl hept-1-yne-1-carboxylate; Methyl pentylacetylenecarboxylate; NSC 72098; Vert de violette artificiel	
Current regulation: Annex III, part 1, n° 89	

had positive reactions to this allergen, tested at only 2% pet. (6). In a previous case report of a fragrance laboratory assistant with work-related ACD both methyl heptin and methyl octin carbonate had been found sensitizers – probably due to their very similar chemical structure (172). In a recent bi-centric study with 350 eczema patients who were consecutively tested with 1% and 2% M20 in pet.; 0.8% positive reactions were observed. However, in 3 additional cases active sensitization, with first reactions appearing 2 to 4 weeks after the patch test, and prompt reactions in the 2 cases repeat-patch tested, was observed (174).

Additional information: /

METHYL EUGENOL	
CAS # 93-15-2	
EC # 202-223-0	
1,2-Dimethoxy-4-(prop-2-enyl)benzene	
4-Allylveratrole; Eugenyl methyl ether extra; 1,2-Dimethoxy-4-allylbenzene; 1,3,4-Eugenol methyl ether; 1-(3,4-Dimethoxyphenyl)-2-propene; 1-Allyl-3,4-dimethoxybenzene; 3,4-Dimethoxy-1-(2-propenyl)benzene; 3,4-Dimethoxyallylbenzene; 3-(3,4-Dimethoxyphenyl)propene; 4-Allyl-1,2-dimethoxybenzene; Benzene, 4-allyl-1,2-dimethoxy-; Chavibetol methyl ether; Ent 21040; Eugenol methyl ether; Eugenyl methyl ether; Methyl eugenol ether; Methyl eugenyl ether; Methylchavibetol; NSC 209528; NSC 8900; O-Methyleugenol; Veratrole methyl ether; Veratrole, 4-allyl-	

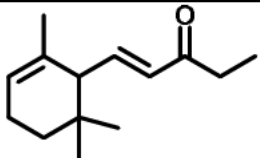
Current regulation: Annex II, 451

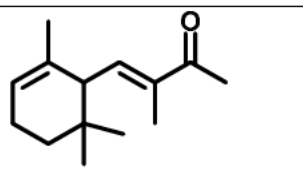
Clinical data:

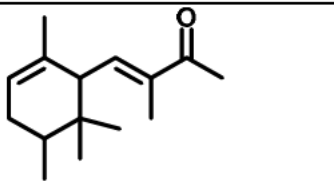
In a previous study by Larsen et al (2002 c), 1.8% of patients with contact allergy to fragrance ingredients reacted positively to this compound (1).

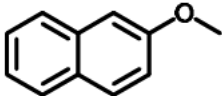
Additional information:

Quote from the SCCS-opinion [0373/00](#): "Methyleugenol should not be intentionally added as a cosmetic ingredient. However, when fragrance compounds containing methyleugenol naturally present in essential oils are used as components in cosmetic products, the highest concentration of methyleugenol in the finished products must not exceed 0.01 % in fine fragrance, 0.004 % in eau de toilette, 0.002 % in a fragrance cream, 0.0002 % in other leave-on products and in oral hygiene products, and 0.001% in rinse-off products." (The reason is genotoxicity and carcinogenicity).

METHYLIONANTHEME	
CAS # 55599-63-8	
EC #	
(1E)-2-Methyl-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-1-penten-3-one mixt. with (3E)-3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	

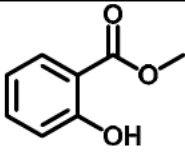
8-Methyl- α -ionone-10-methyl- α -ionone mixt.; Iralia Mixture	
Current regulation: ...	
Clinical	data:
One case of ACD has been reported, caused by an E.d.C. (175).	
Additional information:	
Patented by GIVAUDAN SA 1933, is composed of isomeric n-methylionones and iso-methylionones. Methylionone has CAS # 1335-94-0 (not in CosIng) and 1335-46-2 (METHYL α -IONONE ISOMERS); other names: Methyl- α -cyclocitrilydenacetone; Iralia; Isoaldeine (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.detail&id=41456 , last accessed 2010-07-14).	

5-METHYL-α-IONONE	
CAS # 79-69-6	
EC # 201-219-6	
4-(2,5,6,6-Tetramethyl-2-cyclohexen-1-yl)-3-buten-2-one	
Methyl- α -Ionone; 6-Methyl- α -ionone; α -Irone	
Current regulation: /	
Clinical data:	
In the Frosch 2002 b study, 5 of 1606 consecutive patients (0.3%) showed positive reactions to α -irone (10% pet.) (17).	
Additional information:	
A RIFM review is available (176), citing a (negative) human maximisation test and the study results quoted.	

METHYL beta-NAPHTHYL ETHER	
CAS # 93-04-9	
EC # 202-213-6	
2-Methoxynaphthalene	
beta-Naphthyl methyl ether; methyl 2-naphthyl ether; Nerolin (old); NSC 4171; Yara yara; β-Methoxynaphthalene; β-Naphthol methyl ether; β-Naphthyl methyl ether; 2-Methoxynaphthalene; Methyl β-naphthyl ether; 2-Naphthol methyl ether; 2-Naphthyl methyl ether; 6-Methoxy-2-naphthalene	
Current regulation: /	

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

METHYL SALICYLATE	
CAS # 119-36-8	
EC # 204-317-7	
Methyl 2-hydroxybenzoate	
Other names: Salicylic acid, methyl ester; 2-(Methoxycarbonyl)phenol; 2-Carbomethoxyphenol; 2-Hydroxybenzoic acid methyl ester; Analgit; Anthrapole ND; Ben Gay; Exagien; Flucarmit; Methyl ester of 2-hydroxy benzoic acid; Methyl o-hydroxybenzoate; Methyl salicylate; NSC 8204; Wintergreen oil; o-Hydroxybenzoic acid methyl ester; "Oil of wintergreen"	

Current regulation: /

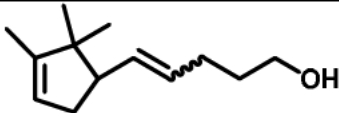
Clinical data:

The deGroot 2000 study yielded 7 positive reactions to methyl salicylate (2% pet.) in 1825 consecutive patients (12).

A case of ACD following the application of a compress bandage containing methyl salicylate has been reported, using 2% "o.o." as PT concentration; the dose per area of methyl salicylate in the occlusive bandage was not reported (177). A similar case was reported in 1977, positive to 2% methyl salicylate in olive oil, with elicitation of pruritus and erythema after oral ingestion of acetyl salicylic acid (178).

Additional information:

A RIFM review is available (179) providing an overview on 3 human sensitisation experiments (e.g., the HRIPT) which were all negative, and clinical data. In a number of older PT studies, positive test results were seen in 6 of 4600, 3 of 183, 3 of 241, 17 of 585, 1 of 70, all employing a test concentration of 2%, usually in pet., according to above review. Methyl salicylate may occur in topical analgesic (OTC) medications, in Germany, for instance, in "Camphopin® Salbe" („Rote Liste 2010").

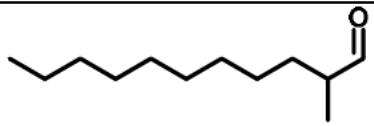
3-METHYL-5-(2,2,3-TRIMETHYL-3-CYCLOPENTENYL)PENT-4-EN-2-OL	
CAS # 67801-20-1	
EC # 267-140-4	
3-Methyl-5-(2,2,3-trimethyl-1-cyclopent-3-enyl)pent-4-en-2-ol	
3-Methyl-5-(2,2,3-trimethyl-3-cyclopenten-1-yl)-4-penten-2-ol; 3-Methyl-5-(2,2,3-trimethylcyclopent-3-enyl)pent-4-en-2-ol; Ebanol	

Current regulation: /

Clinical data:

In the Larsen 2001 study, 1 of 178 patients with known contact allergy to fragrance ingredients exhibited a positive PT reaction to "MTCP", tested 5% pet. (19). In the An 2005 study, 12 of 422 consecutive patients, i.e., 2.8%, had positive reactions to "ebanol", tested at 5% (13).

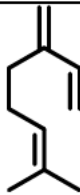
Additional information: /

METHYLUNDECANAL	
CAS # 110-41-8	
EC # 203-765-0	
2-Methylundecanal	
Aldehyde c-12 mna; undecenal, 2-methyl-; 2-Methyl-1-undecanal; Aldehyde M.N.A.; Methyl n-nonyl acetaldehyde; Methylnonylacetaldehyde; NSC 46127	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

MYRCENE	
CAS # 123-35-3	
EC # 204-622-5	
7-Methyl-3-methylideneocta-1,6-diene	
2-Methyl-6-methylene-2,7-octadiene; 7-Methyl-3-methylene-1,6-octadiene; NSC 406264; β-Geraniolene; β-Myrcene	

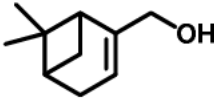
Current regulation: /

Clinical data:

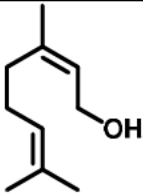
In a clinical study in 6 European centres, including 1511 consecutive patients, 1 patient had a positive reaction to oxidized myrcene (65).

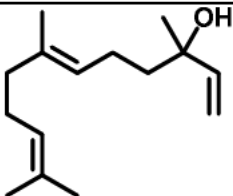
Additional information:

Myrcene autoxidizes spontaneously and rapidly at air exposure. In experimental studies on beta-myrcene an EC3 value of 4.3% was seen for a sample air-exposed 10 weeks (Sköld M. Contact allergy to autoxidized fragrance terpenes (180).

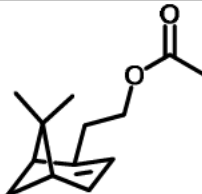
MYRTENOL	
CAS # 515-00-4	
EC # 208-193-5	

(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)methanol
(-)-Pin-2-ene-10-ol; 2-Pinen-10-ol; (6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methanol; (±)-Myrtenol; 6,6-Dimethyl-2-(hydroxymethyl)bicyclo[3.1.1]hept-2-ene; NSC 408846; α-Pinene-10-ol
Current regulation: /
Clinical data: /
Additional information:
A RIFM review exists (181), citing 2 of 3 HRIPT studies with 1 case of sensitisation to myrtenol each.

NEROL	
CAS # 106-25-2	
EC # 203-378-7	
(2Z)-3,7-Dimethylocta-2,6-dien-1-ol	
2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-; (Z)-3,7-Dimethyl-2,6-octadien-1-ol; (Z)-Geraniol; (Z)-Nerol; 2-cis-3,7-Dimethyl-2,6-octadien-1-ol; 3,7-Dimethyl-cis-2,6-octadien-1-ol; Nerol 900; Neryl alcohol; cis-3,7-Dimethyl-2,6-octadien-1-ol; cis-Geraniol; β-Nerol; cis-geraniol – i.e., isomeric to geraniol	
Current regulation: /	
Clinical data:	
In the Larsen 2002 c study, 6.0% of the fragrance sensitive patients reacted positively to 5% in pet. (1).	
Additional information:	
A RIFM review is available (182) citing (negative) human sensitisation experiments, an older study from Japan and the Larsen 2002 c study (see above).	
Regarding autoxidation studies – see geraniol.	

Nerolidol (isomer not specified)	
CAS # 7212-44-4	
EC # 230-597-5	
3,7,11-Trimethyl-1,6,10-odecatrien-3-ol	
Nerolidol; (±)-Nerolidol; FCI 119b; Nerodilol	
Current regulation: /	
Clinical data: /	
Additional information:	

RIFM review is available (183) citing the occurrence of "3 positive reactions in 2273 patients". Another RIFM review is available on cis-nerolidol (184), mentioning that no data on this compound are available.

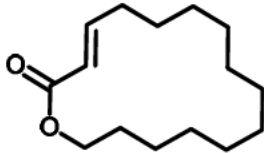
NOPYL ACETATE	
CAS # 128-51-8	
EC # 204-891-9	
2-(7,7-Dimethyl-4-bicyclo[3.1.1]hept-3-enyl)ethyl acetate	
2-Norpinene-2-ethanol, 6,6-Dimethyl-, acetate; Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-, acetate; 2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl acetate; 7,7-Dimethylbicyclo[3.1.1]hept-2-ene-2-ethanol acetate; Citroviol; NSC 1286; NSC 404963; Nopol acetate; Nopyl acetate	

Current regulation: /

Clinical data:

The DeGroot 1985 study identified 2 (1.1%) positive reactions among 179 patients using a 25% PT preparation of this compound – reactions may have at least partly been due to an "excited back syndrome" and thus a limited evidence (25).

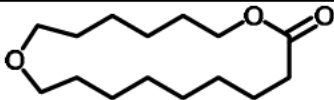
Additional information: /

OXACYCLOHEXADECENONE	
CAS # 34902-57-3	
EC # 609-040-9	
(3E)-Oxacyclohexadec-3-en-2-one	
Globalide; Oxacyclohexadecen-2-one	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

OXALIDE	
CAS # 1725-01-5	
EC # 217-033-3	
1,8-Dioxacycloheptadecan-9-one	
Nonanoic acid, 9-[(6-hydroxyhexyl)oxy]-, o-lactone; 10-Oxa-16-hexadecanolide; Oxalide; Oxalide T	

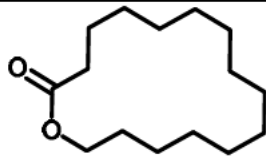
Current regulation: /

Clinical data:

In the Larsen 2001 study, none of 178 patients with known contact allergy to fragrance ingredients exhibited a positive PT reaction to "10-oxahexadecanolide", tested 5% pet. (19).

Additional information:

A RIFM review is available (128), citing a negative maximisation test (n=29).

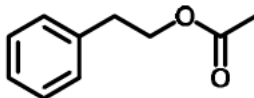
PENTADECALACTONE	
CAS # 106-02-5	
EC # 203-354-6	
1-Oxacyclohexadecan-2-one	
Pentadecanoic acid, 15-hydroxy-, ξ -lactone; 1,15-Pentadecanolide; 15-Hydroxypentadecanoic acid lactone; 15-Pentadecanolide; 15-Pentadodecanolactone; 2-Pentadecalone; CPE 215; Cyclopentadecanolide; Exaltolide; Macrolide Supra; Muskalactone; NSC 36763; Pentadecalactone; Pentadecanolactone; Pentadecanolide; Pentalide; Thibetolide; cpd Supra; ω -Pentadecalactone; angelica lactone; hexaltolide	

Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance (IFRA, pers. comm.2010). The substance has been used for clinical olfactory testing in the 60ies under the name of exaltolide.

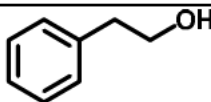
PHENETHYL ACETATE	
CAS # 103-45-7	
EC # 203-113-5	
2-Phenylethyl acetate	
Acetic acid, phenethyl ester ; Phenethyl alcohol, acetate; 2-Phenethyl acetate; 2-Phenylethyl acetate; Benzylcarbiny acetate; NSC 71927; Phenethyl acetate; Phenylethyl ethanoate; β -Phenethyl acetate; β -Phenylethanol acetate; β -Phenylethyl acetate	

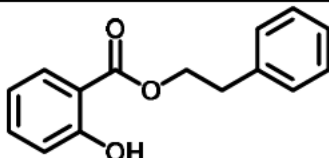
Current regulation: /

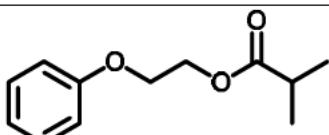
Clinical data: /

Additional information:

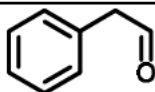
It is a "top 100" substance (IFRA, pers. comm.2010). Exposure via plants (*Tanacetum parthenium*) is possible (185).

PHENETHYL ALCOHOL	
CAS # 60-12-8	
EC # 200-456-2	
2-Phenylethanol	
Phenethyl alcohol; (2-Hydroxyethyl)benzene; 2-Phenethanol; 2-Phenethyl alcohol; 2-Phenyl-1-ethanol; 2-Phenylethyl alcohol; Benzyl carbinol; Ethanol, 2-phenyl-; NSC 406252; PEA; Phenethanol; Phenethylol; Phenylethanol; Phenylethyl alcohol; β -(Hydroxyethyl)benzene; β -PEA; β -Phenethanol; β -Phenethyl alcohol; β -Phenethylol; β -Phenylethanol; β -Phenylethyl alcohol	
Current regulation: /	
Clinical data:	
<p>The DeGroot 1985 study identified 1 (0.6%) positive reactions among 179 patients using a 25% PT preparation of phenylethyl alcohol (25). In the Frosch 1995 dose-finding pilot study, no positive reaction to this compound, tested 1% pet. in 100 consecutive patients in Odense, DK, was observed (15).</p>	
Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).	

PHENETHYL SALICYLATE	
CAS # 87-22-9	
EC # 201 732-5	
2-Phe ylethyl 2-hydroxybenzoate	
Salicylic acid, phenethyl ester; 2-Phenylethyl salicylate; Benzylcarbinyl salicylate; NSC 72035; Phenethyl salicylate	
Current regulation: /	
Clinical data: /	
Additional information:	
A RIFM review exists (186), quoting a negative human maximisation test and a number of animal experiments, including cross-sensitisation experiments with benzyl salicylate. One LLNA study is reported yielding an EC3 value of 2.1%.	

PHENOXYETHYL ISOBUTYRATE	
CAS # 103-60-6	
EC # 203-127-1	
2-Phenoxyethyl 2-methylpropanoate	
Isobutyric acid, 2-phenoxyethyl ester; Ethanol, 2 phenoxy , isobutyrate; 2-Phenoxyethyl isobutyrate; NSC 227210; NSC	

406209; Phenoxyethyl isobutyrate; β -Phenoxyethyl isobutyrate	
Current regulation: /	
Clinical data: /	
Additional information:	
It is a "top 100" substance (IFRA, pers. comm.2010).	

PHENYLACETALDEHYDE	
CAS # 122-78-1	
EC # 204-574-5	
2-Phenylacetaldehyde	
Benzylcarboxaldehyde; Hyacinthin; NSC 406309; Phenacetaldehyde; Phenylacetaldehyde; Phenylacetic aldehyde; Phenylethanal; α -Phenylacetaldehyde; α -Tolualdehyde; α -Toluic aldehyde	

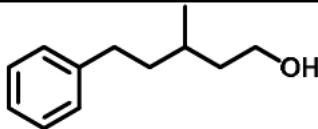
Current regulation: /

Clinical data:

In the Malten 1984 study, 1.1% of 182 patients displayed a positive PT reaction to phenylacetaldehyde 2% pet. (24). In a case report, Sanchez-Politta et al. describe a 26-year-old worker in a perfume factory, who suffered from a spill of pure phenylacetaldehyde and became sensitised, as proven by positive patch tests with 0.5%, 1% and 2% (10 healthy controls negative) (187).

Additional information:

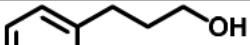
SCCS opinion: [1153/08 - Opinion on "Dermal Sensitization Quantitative Risk Assessment" \(QRA: Citral, farnesol and phenylacetaldehyde\)](#)

PHENYLISOHEXANOL	
CAS # 55066-48-3	
EC # 259-461-3	
3-Methyl-5-phenylpentan-1-ol	
3-Methyl-5-phenyl-1-pentanol; 3-Methyl-5-phenylpentanol; 5-Phenyl-3-methylpentanol; Mefrosol; Phenoxanol	

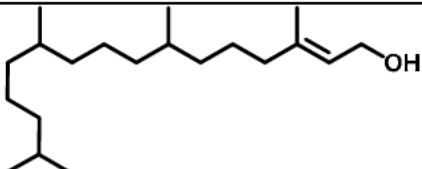
Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

PHENYLPROPANOL	
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CAS # 122-97-4
EC # 204-587-6
3-Phenylpropan-1-ol
(3-Hydroxypropyl)benzene; 1-Hydroxy-3-phenylpropane; 3-Benzenepropanol; 3-Hydroxy-1-phenylpropane; 3-Phenyl-1-propanol; 3-Phenyl-n-propanol; 3-Phenylpropanol; 3-Phenylpropyl alcohol; Dihydrocinnamyl alcohol; Hydrocinnamic alcohol; Hydrocinnamyl alcohol; NSC 16942; γ-Phenylpropanol; γ-Phenylpropyl alcohol; Phenethyl Carbinol
Current regulation: /
Clinical data:
The Larsen 2002 c study yielded 0.9% positive reactions in 218 patients with contact allergy to fragrance ingredients (1).
Additional information: ...

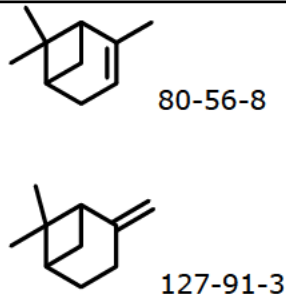
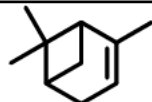
PHYTOL	
CAS # 150-86-7	
EC # 205-776-6	
(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	
Phytol; (7R,11R,2E)-Phytol; (E)-Phytol; (E,R,R)-Phytol; 3,7,11,15-Tetramethylhexadec-2-en-1-ol; trans-Phytol	

Current regulation: /

Clinical data:

Additional information:

Phytol is a main constituent of Jasmin abs. with 7.4% reported content (17). In a human maximization study involving 25 subjects, there was one case of contact sensitization to 10% phytol (6900 µg/cm²), applied in petrolatum, as reported in a RIFM review (188).

alpha-PINENE and beta-PINENE	
CAS # 80-56-8 (alpha-Pinene); CAS # 127-91-3 (beta-Pinene)	
EC # 201-291-9 (alpha-Pinene; according to CAS service: 219-445-9); EC # 204-872-5 (beta-Pinene; according to CAS service: 245-424-9)	
2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (80-56-8)	
6,6-Dimethyl- 2-methylenebicyclo[3.1.1]heptane (127-91-3)	

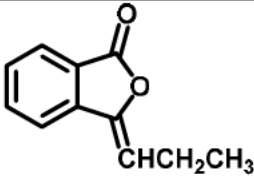
80-56-8: 2-Pinene; (\pm)-2-Pinene; (\pm)- α -Pinene; Acintene A; NSC 7727; PC 500; PC 500 (terpene); Sylvapine A; α -Pinene 127-91-3: 2(10)-Pinene ; (\pm)-2(10)-Pinene; (\pm)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; (\pm)- β -Pinene; 6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane; NSC 21447; NSC 406265; NSC 59190; Nopinen; Nopinene; PC 600; PC 600 (pesticide); Pseudopinen; Pseudopinene; Terebenthene; β -Pinene	
Current regulation: Annex III, part 1, n° 130 (Peroxide value less than 10 mmol/L in substance)	

Clinical data:

In 63 patients positive to the FM I, 2 had a positive PT reaction to beta-pinene (and none to alpha-pinene 5% pet.), 1% pet., in the Santucci 1987 study (28). A clinical series from Portugal, addressing contact allergy to oil of turpentine diagnosed in 30 patients, used a series with pure terpenes. A total of 17 of 30 patients reacted positively to alpha-pinene, and 2 to beta-pinene (189). In a series of 24 patients with occupational contact dermatitis from the pottery industry, Lear et al. found 14 to be sensitised to "Indonesian oil of turpentine" and 8 to alpha-pinene (190).

A case report from Zacher and Ippen on 2 patients with allergic contact dermatitis due to bergamot oil (191) describes positive patch test reactions to alpha-pinene and beta-pinene in one, a worker in a perfume factory.

Additional information: /

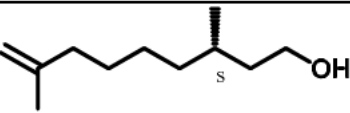
PROPYLIDENE PHTHALIDE	
CAS # 17369-59-4	
EC # 241-402-8	
3-Propylidene-2-benzofuran-1-one	
3-Propylidene-1(3H)-isobenzofuranone; Propylidenephthalide; Celeriax; Propylidenephthalide	3-

Current regulation: Annex III, part 1, n° 175

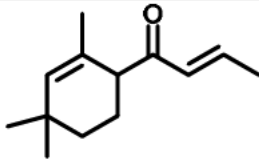
Clinical data:

In the Malten 1984 study, 2.6% of 182 patients displayed a positive PT reaction to ethyl acrylate 1% pet. (24). In this paper, "3/25" positive results in human maximisation tests are listed.

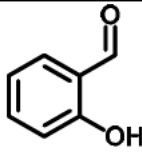
Additional information: /

RHODINOL	
CAS # 6812-78-8	
EC # 229-887-4	
(3S)-3,7-Dimethyloct-7-en-1-ol	
Rhodinol; (-)-Rhodinol; α -citronellol; (-)- α -Citronellol; (S)-	

α-Citronellol	
Current regulation: /	
Clinical data: / (see below)	
Additional information:	
A RIFM review exists citing a positive HRIPT with several cases of sensitisation, 5 of these proven upon re-challenge, and a negative human maximisation test (192). In a previous RIFM review (128), a Japanese clinical study (source not accessible) is cited: "In patch tests using cosmetics ingredients and fragrance materials on patients with eczema and dermatitis, 5% rhodinol (vehicle not specified) produced one sensitization reaction in 202 patients (Itoh et al., 1988 ¹⁴)"	

<i>trans</i>-ROSE KETONE-5	
CAS # 39872-57-6	
EC # 254-663-8	
(2E)-1-(2,4,4-Trimethylcyclohex-2-en-1-yl)but-2-en-1-one	
alpha-Isodamascone; trans-2,4,4-Trimethyl-1-crotonyl-2-cyclohexene; (E)-1-(2,4,4-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one	
Current regulation: Annex III, part 1, n° 159 (max. conc. 0.02%)	

Clinical data: /
Additional information:
A RIFM review is available (193) quoting 2 HRIPT studies: one with 0.2% concentration in DEP in 103 volunteers, and negative result, one with 2% concentration, sensitising 2 of 22 volunteers.

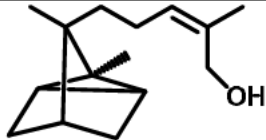
SALICYLALDEHYDE	
CAS # 90-02-8	
EC # 201-961-0	
2-Hydroxybenzaldehyde	
Salicylaldehyde; 2-Formylphenol; NSC 112278; NSC 49178; NSC 83559; NSC 83560; NSC 83561; NSC 83562; NSC 97202; Salicylal; Salicylic aldehyde; o-Formylphenol; o-Hydroxybenzaldehyde	
Current regulation: /	

Clinical data:

¹⁴ Itoh M., Hosono K., Kantoh H., Kinoshita M., Yamada K., Kurosaka R. and Nishimura M. (1988) Patch test results with cosmetic ingredients conducted between 1978-1986. *Nippon Koshohen Kagakkaishi* 12 (1), 27-41.

In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to salicylaldehyde was observed (3). In the Wöhr 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reaction to salicylaldehyde 2% pet. (22). The IVDK 2010 study, 0.48% (95% CI: 0.18 – 0.79%; percentages standardised for age and sex) of 2729 patients PTed reacted to the compound (7). An earlier study by Bruze and Zimerson points to possible cross-reactivity between salicylaldehyde and “simple methylol phenols” occurring in synthetic resins based on phenol and formaldehyde (194). Among 24 patients sensitised to resorcinol by application of a wart remover, 2 positive reactions to salicylaldehyde were observed (195).

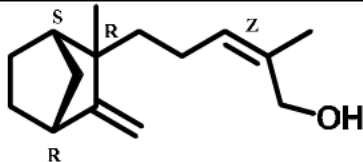
Additional information: Along with other derivates of salicylic acid, salicylaldehyde is found in the bark of several trees, such as willow or aspen, and can cause allergic contact dermatitis by this exposure (196).

<i>alpha-SANTALOL</i>	
CAS # 115-71-9	
EC # 204-102-8	
(R Z)- 5-(2,3-dimethyltricyclo[2.2.1.0^{2,6}]hept-3-yl)-2-methylPent-2-en-1-ol	
2-Penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.0 ^{2,6}]hept-3-yl)-2-methyl-, [R(Z)]-; 2-Penten-1-ol, 5-(2,3-dimethyltricyclo[2.2.1.0 ^{2,6}]hept-3-yl)-2-methyl-, stereoisomer; α-Santalol; Tricyclo[2.2.1.0 ^{2,6}]heptane, 2-penten-1-ol deriv.; (+)-(Z)-α-Santalol; (+)-α-Santalol; (Z)-α-Santalol; Sandal; Santalol a; cis-α-Santalol; d-α-Santalol	

Current regulation: /

Clinical data: / (see beta-santalol)

Additional information:
 Following a precautionary principle, both isoforms – often not differentiated in reports – are considered as one and considered as established contact allergen in humans.

<i>beta-SANTALOL</i>	
CAS # 77-42-9	
EC # 201-027-2	
(2Z)-2-Methyl-5-[(1S,2R,4R)-2-methyl-3-methylenebicyclo[2.2.1]hept-2-yl]pent-2-en-1-ol	
2-Methyl-5-(2-methyl-3-methylene-2-norbornyl)-2-penten-1-ol; [1S-[1α,2α(Z),4α]]-2-Methyl-5-(2-methyl-3-methylenebicyclo[2.2.1]hept-2-yl)-2-penten-1-ol; β-Santalol; (-)-(Z)-β-Santalol; (-)-β-Santalol; Santalol b; cis-β-Santalol	

Current regulation: /

Clinical

data:

A RIFM review is available for alpha-santalol (197) and on "santalol" (CAS # 11031-45-1 (198)). The former review cites a Japanese study: "Between April 1979 and August 1990, a total of 3123 male and female patients were patch tested to 2% santalol (.alpha. or .beta. not specified) in petrolatum. Reactions were observed in 47/3123 (1.5%) of the patients. The incidence of positive reactions from 1979 to 1990 was 1.5%. The rate of reactions observed was higher during the earlier period of the patch testing than the later stage (Utsumi et al., 1992)¹⁵." In another Japanese study cited by the RIFM review "... patch tests were conducted with 0.05–0.5% santalol (specified as santalol 1) in a base cream or in 99% ethanol. Patches consisted of a piece of 1 cm² lint with a 2 cm² cellophane disc placed on the lint and then covered with a 4 cm² plaster. Patches were applied to the back, the forearm, and the inside of the upper arm for 24–48 h. Reactions were observed in 15 patients and questionable reactions were observed in 10 patients out of the total 427 participating. A second sample of santalol (specified as santalol 2) was tested on 214 patients. Reactions were observed in three patients and questionable reactions were observed in six patients (Takenaka et al., 1986)¹⁶." Moreover, "The Mid-Japan Contact Dermatitis Research group (MJDCRG) conducted a 6-year (1976–1981) patch test study on facial dermatoses patients with various fragrance materials. During the year 1979, a total of 327 patients were tested with a mixture of .alpha. and .beta. santalol at concentrations of 10%, 2%, and 1% in white petrolatum. Reactions were observed in 1.5%, 0.6% and 0.6% of the 327 patients tested at concentrations 10%, 2%, and 1%, respectively (MJDCRG, 1984)¹⁷."

The Goossens 1997 study found 5 of 111 patients positive to "santalol 10% pet." (isoform not specified) – all sensitised to other fragrance allergens as well (23). In the Larsen 2001 study, patch testing with "2-methyl-5-(2,3-dimethyltricyclo[2.2.1.0(2,6)]hept-3-yl-2-pentenol(.alpha.-form) and 2-methyl-5-(2-methyl-3-methylenebicyclo[2.2.1]hept-3-yl-2-penten-1-ol(beta-form) 5% pet." (no CAS numbers given) yielded a total of 2 positive reactions among the 178 patients with known contact allergy to fragrance ingredients (19).

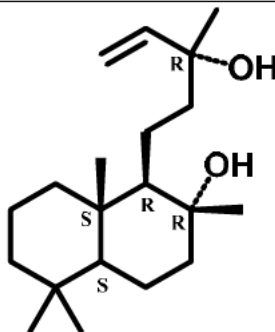
Additional information: "There is no one CAS number for the mixture. The alpha form has a CAS No. 115-71-9 and the beta form is 37172-32-0 (this # is trans-.beta.-santalol). There was no reported use of these materials in the last two IFRA Surveys (8 years total)" (A.M. Api, pers. comm., 2010).

Following a precautionary principle, both isoforms – often not differentiated in reports – are considered as one and considered as established contact allergen in humans

¹⁵ Utsumi, M., Sugai, T., Shoji, A., Watanabe, K., Asoh, S., Hashimoto, Y., 1992. Incidence of positive reactions to sandalwood oil and its related fragrance materials in patch tests and a case of contact allergy to natural and synthetic sandalwood oil in a museum worker. *Skin Research* 34, 209–213

¹⁶ Takenaka, T., Hasegawa, E., Takenaka, U., Saito, F., Odaka, T., 1986. Fundamental studies of safe compound perfumes for cosmetics Part 1. The primary irritation of compound materials to the skin. *Unknown Source*, 313–329.

¹⁷ Mid-Japan Contact Dermatitis Research Group, 1984. Determination of suitable concentrations for patch testing of various fragrance materials. A summary of group study conducted over a 6-year period. *Journal of Dermatology*, 11(1), 31–35.

SCLAREOL	
CAS # 515-03-7	
EC # 208-194-0	
(1R,2R,8aS)-1-[(3R)-3-Hydroxy-3-methylpent-4-enyl]-2,5,5,8a-tetramethyl-3,4,4a,6,7,8-hexahydro-1H-naphthalen-2-ol	
(αR,1R,2R,4aS,8aS)-α-Ethenyldecahydro-2-hydroxy-α,2,5,5,8a-pentamethyl-1-naphthalenepropanol; [1R-[1α(R*),2β,4αβ,8α]] - α-ethenyldecahydro-2-hydroxy-α,2,5,5,8a-pentamethyl-1 Naphthalenepropanol; (13R)-Labd-14-ene-8,13-diol; Sclareol; (-)-Sclareol; [1R-[1.α.(R*),2.β.,4α.β.,8α.α.]]-2-hydroxy-α,2,5,5,8a-pentamethyl-α-vinyldecahydronaphthalene-1-propan-1-ol	

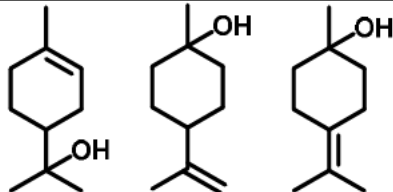
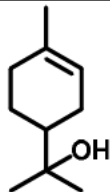
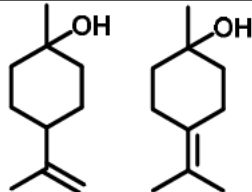
Current regulation: /

Clinical data: /

Additional information:

An older RIFM review exists (128), reporting several human maximisation tests with different samples of sclareol, yielding partly positive, partly negative results. A more recent RIFM review is available (199), citing no clinical data, but several maximisation studies, one of which was positive in a few volunteers, which was apparently due to an impurity.

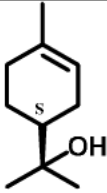
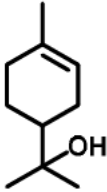
[0986/06 - Opinion on Sclareol \(sensitisation only\)](http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_056.pdf)
(http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_056.pdf)

TERPINEOL	
CAS # 8000-41-7	
EC # 232-268-1	
Mixtures of isomers	
Terpineol 318, mixture of terpineol isomers alfa, beta, gamma	<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  alfa gamma </div> <div style="text-align: center;">  beta </div> </div>

Current regulation: /

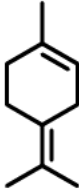
Clinical data:
A RIFM review is available (200), citing negative human induction studies and one clinical study "Takenaka 1986", finding 4 of 312 patients with 0.05% to 0.5% terpineol in a cream base and in ethanol, resp., and 2 negative clinical studies of limited size. In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% terpineol in pet., tested in 100 consecutive patients in Belfast, were observed (15).

Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010).

<i>alpha-TERPINEOL</i>	 10482-56-1
CAS # 10482-56-1 / 98-55-5	
EC # 233-986-8 / 202-680-6	
2-[(1S)-4-Methyl-1-cyclohex-3-enyl]propan-2-ol (10482-56-1)	
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-ol (98-55-5)	
10482-56-1: (S)-(-)-p-Menth-1-en-8-ol; (-)- α -Terpineol; (S)-(-)-Terpineol; (S)-(-)- α -Terpineol; (S)- α -Terpineol; l- α -Terpineol 98-55-5: p-Menth-1-en-8-ol; (\pm)- α -Terpineol; 1,1-Dimethyl-1-(4-methylcyclohex-3-enyl)methanol; 1-p-Menthen-8-ol; 2-(4-Methyl-3-cyclohexenyl)-2-propanol; 4-(2-Hydroxy-2-propyl)-1-methylcyclohexene; 8-Hydroxy-p-menth-1-ene; NSC 21449; NSC 403665; PC 593; Pine Oil 593; Terpineol 350; dl- α -Terpineol; $\alpha,\alpha,4$ -Trimethyl-3-cyclohexene-1-methanol; α -Terpineol	 98-55-5
Current regulation: /	

Clinical data:
 A RIFM review is available (201) specifically on (-)-alpha-terpineol stating that "no data is available" regarding skin sensitisation. Another RIFM review is available on alpha-terpineol (202). In the Frosch 2002 b study, 1 of 1606 consecutive patients showed a positive reaction, but 11 patients doubtful reactions to alpha-terpineol (5% pet.) (17). The DeGroot 1985 study identified no positive reactions among 179 patients using a 15% PT preparation of terpineol (mixed isomers) (25). In 63 patients positive to the FM I, 2 had a positive PT reaction to alpha terpineol, 5% pet., in the Santucci 1987 study (28). A clinical series from Portugal, addressing contact allergy to oil of turpentine diagnosed in 30 patients, used a series with pure terpenes. A total of 3 of 30 patients reacted positively to alpha-terpineol (189)

Additional information: see also terpineol (mixture of isomers). Comments on turpentine under pinene.

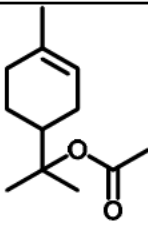
<i>Terpinolene</i>	
CAS # 586-62-9	
EC # 209-578-0	
1-Methyl-4-propan-2-ylidenecyclohexene	
p-Mentha-1,4(8)-diene; 1-Methyl-4-(1-methylethylidene)-cyclohexene; 4-Isopropylidene-1-methylcyclohexene; Isoterpinene; Nofmer TP; Terpinolen; Terpinolene; α -Terpinolene; δ -Terpinene	

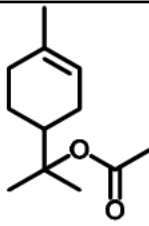
Current regulation: Annex III, part 1, n° 133 (Peroxide value less than 10 mmoles/L in substance)

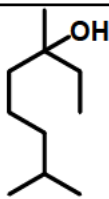
Clinical data:
 A 49-year-old machine cleaner developed occupational contact dermatitis due to the cleaner, which gave a positive patch test result at 1:10 000 in water. Of the ingredients identified by chromatography, only δ -3-carene and terpinolene, tested 5% pet.,

gave a positive result (negative in 10 controls) (203). Eleven patients sensitised to tea tree oil showed positive reactions to alpha-terpinene, terpinolene and ascaridol (204).

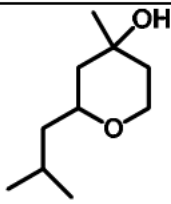
Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010)

TERPINEOL ACETATE (Isomer mixture)	
CAS # 8007-35-0	
EC # 232-357-5	
4-Methyl-1-propan-2-yl-1-cyclohex-2-enyl acetate	
Terpinyl acetate	
Current regulation: /	
Clinical	data:
In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% terpinyl acetate in pet., tested in 106 consecutive patients in Barcelona, were observed (15)	
Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010)	

alpha-TERPINYL ACETATE	
CAS # 80-26-2	
EC # 201-265-7	
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-yl acetate	
3-Cyclohexene-1-methanol, α,α,4-trimethyl-, acetate; p-Menth-1-en-8-ol, acetate; (±)-α-Terpineol acetate; (±)-α-Terpinyl acetate; 2-(4-Methyl-3-cyclohexen-1-yl)-2-propyl acetate; Terpinyl acetate; α-Terpineol acetate; p-Menth-1-en-8-yl acetate; 1-Methyl-1-(4-methylcyclohex-3-enyl)ethyl ethanoate; (±)-.alpha.,.alpha.,4-trimethylcyclohex-3-ene-1-methyl acetate	
Current regulation: /	
Clinical data:	
The DeGroot 1985 study identified no positive reactions among 179 patients using a 10% PT preparation of "terpinyl acetate" (25).	
Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010)	

Tetrahydrolinalool	
CAS # 78-69-3	
EC # 201-133-9	
3,7-Dimethyloctan-3-ol	
2,6-Dimethyl-6-octanol; 3,7-Dimethyloctan-3-ol; Linalool tetrahydride; NSC 128151; Tetrahydrolinalool	
Current regulation: /	
Clinical	data:
/	
Additional information: It is a “top 100” substance (IFRA, pers. comm. 2010). A RIFM	

review is available (205) quoting 1 negative human maximisation test.

TETRAHYDRO-METHYL-METHYLPROPYL)-PYRAN-4-OL	
CAS # 63500-71-0	
EC # 405-040-6	
4- Methyl-2-(2-methylpropyl)tetrahydro-2H-4-pyranol	
2-(2-Methylpropyl)-4-hydroxy-4-methyltetrahydropyran; 2-Isobutyl-4-hydroxy-4-methyltetrahydropyran; 2-Isobutyl-4-methyltetrahydropyran-4-ol; 4-Hydroxy-4-methyl-2-(2-methylpropyl)tetrahydropyran; Florosa; Rozanol	

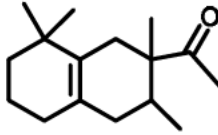
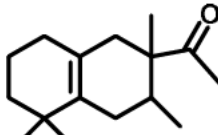
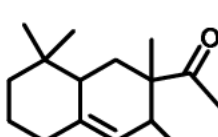
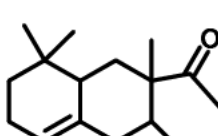
Current regulation: /

Clinical

data:

/

Additional information: It is a "top 100" substance (IFRA, pers. comm.2010).

TETRAMETHYL ACETYLOCTAHYDRONAPHTHALENES	
CAS # 54464-57-2 / 54464-59-4 / 68155-66-8 / 68155-67-9	
EC # 259-174-3 / 259-175-9 / 268-978-3 / 268-979-9	
1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (54464-57-2)	
1-(1,2,3,4,5,6,7,8-Octahydro-2,3,5,5-tetramethyl-2-naphthalenyl)-ethanone (54464-59-4)	
1-(1,2,3,5,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (68155-66-8)	
1-(1,2,3,4,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)-ethanone (68155-67-9)	
54464-57-2: 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthalenyl)ethanone; 1',2',3',4',5',6',7',8'-Octahydro-2',3',8',8'-tetramethyl-2'-acetoneaphthone; 7-Acetyl-1,2,3,4,5,6,7,8-octahydro-1,1,6,7-tetramethylnaphthalene; Amberonne; Ambralux; Iso Ambois Super; Iso-E Super; Isocyclemon E; OTNE; Orbitone	

Current regulation: /

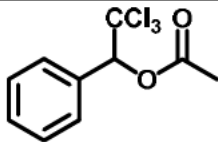
Clinical

data:

In the Frosch 2002 a study, 0.2% of 1855 consecutive patients reacted to the compound (brand name mentioned: „Iso E. Super“, 5% pet.) (16). In the Frosch 1995 dose-finding pilot study, 1 positive reaction both to 1% and 5% "Iso E Super ®" in pet., tested in 313 consecutive patients in Bordeaux and London, were observed (15). The Larsen 2001 study yielded 1.7% positive reactions (5% pet.) in 178 patients with known contact allergy to fragrance ingredients (19).

Additional information: According to CosIng: "Mixture of isomers: 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one; 1-(1,2,3,4,5,6,7,8-Octahydro-2,3,5,5-tetramethyl-2-naphthyl)ethan-1-one; 1-(1,2,3,5,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one (68155-67-9); 1-(1,2,3,4,6,7,8,8a-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one (68155-66-8) "
<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40504>, last accessed 2009-11-11).

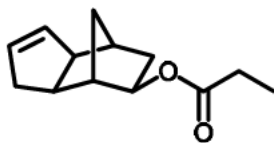
It is a "top 100" substance (IFRA, pers. comm. 2010)

TRICHLOROMETHYL PHENYL CARBINYL ACETATE	
CAS # 90-17-5	
EC # 201-972-0	
2,2,2-Trichloro-1-phenylethyl acetate	
Benzenemethanol, α-(trichloromethyl)-, acetate; Benzyl alcohol, α-(trichloromethyl)-, acetate (Trichloromethyl)phenylcarbinyl acetate; (±)-α-(Trichloromethyl)benzyl acetate; 2-Acetoxy-1,1,1-trichloro-2-phenylethane; Crystal rose; NSC 165582; Rosacetol; Rosephenone; Rosetone; Rosone; α-(Trichloromethyl)benzyl acetate	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010)

TRICYCLODECENYL PROPIONATE	
CAS # 17511-60-3	
EC # 241-514-7	
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-inden-6-yl propionate	
4,7-Methano-1H-inden-6-ol, 3a,4,5,6,7,7a-Hexahydro-, propanoate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-Hexahydro-, propionate; Cyclaprop; Florocyclene; Greenyl propionate; Tricyclo(5.2.1.02,6)dec-3-en-8-yl propionate.	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010).

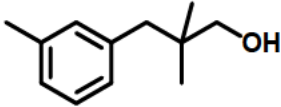
3-(5,5,6-TRIMETHYLBICYCLO[2.2.1]HEPT-2-YL)-CYCLOHEXAN-1-OL	
---	---

CAS # 3407-42-9	
EC # 222-294-1	
3-(5,5,6-Trimethyl-6-bicyclo[2.2.1]heptanyl)cyclohexan-1-ol	
3-(5,5,6-Trimethyl-2-norbornyl)-cyclohexanol; 3-(5,5,6-Trimethylbicyclo[2.2.1]hept-2-yl)cyclohexan-1-ol; 3-Hydroxy-1-(5-isocampyl)cyclohexane; Sandela	

Current regulation: /

Clinical data: /

Additional information: part of "synthetic sandalwood oil".

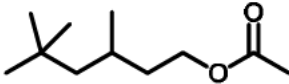
TRIMETHYL-BENZENEPROPANOL (Majantol)	
CAS # 103694-68-4	
EC # 403-140-4	
2,2-Dimethyl-3-(3-methylphenyl)propan-1-ol	
2,2-Dimethyl-3-(3-tolyl)propan-1-ol; 3-(2,2-Dimethyl-3-hydroxypropyl)toluene	

Current regulation: /

Clinical data:

In the Larsen 2002 c study, majantol (conc. not given, elsewhere reported as 5% pet.) caused positive PT reactions in 3.2% of patients with known contact allergy to fragrance ingredients. In a later study by the IVDK, 0.5% (95% CI: 0.3 – 0.7%) consecutive patients displayed a positive reaction to majantol 5% pet. (206). In the IVDK 2010 study, majantol was tested both in n=2189 consecutive patients, yielding 0.36 % (95% CI: 0.12–0.60%) positive reactions, and in the context in a special series, applied in an aimed fashion to n=4972 patients, yielding 0.76% (95% CI: 0.49–1.03%) (standardised) positive reactions (7). In a recent study from Copenhagen, DK, 6 of 722 patients tested with this compound were found positive, 2 of these to material used earlier provided by Symrise, 4 to material by Allmiral/Hermal/Trolab used later instead. There was no significant difference between these proportions obtained with batches of majantol from different production processes (207).

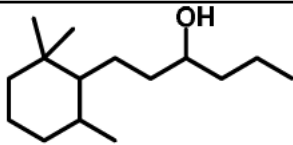
Additional information: /

TRIMETHYLHEXYL ACETATE	
CAS # 58430-94-7	
EC # 261-245-9	
3,5,5-Trimethylhexyl acetate	
1-Hexanol, 3,5,5-trimethyl-, acetate; Vanoris; neononyl acetate	

Current regulation: /

Clinical data: /

Additional information: It is a "top 100" substance (IFRA, pers. comm. 2010)

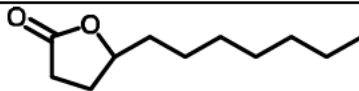
TRIMETHYL-PROPYLCYCLOHEXANEPROPANOL (TMCH)	
CAS # 70788-30-6	
EC # 274-892-7	
1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol	
Other names: 2,2,6-Trimethyl-alpha-propylcyclohexanepropanol (REACH, EINECS); .alpha.-Propyl-2,2,6-trimethylcyclohexanepropanol; 6-(2,2,6-Trimethylcyclohexyl)-4-hexanol; Finotimber; Timberol	

Current regulation: /

Clinical data:

In the Larsen 2001 study, none of 178 patients with contact allergy to fragrance ingredients reacted positively to this ingredient, PTed at 5% pet. (19).

Additional information: /

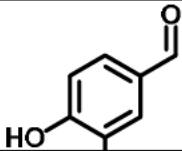
<i>gamma</i>-UNDECALACTONE	
CAS # 104-67-6	
EC # 203-225-4	
5-Heptyltetrahydrofuran-2-one	
Undecanoic acid, 4-hydroxy-, γ-lactone; (RS)-γ-Undecalactone; (±)-γ-Undecalactone; 4-Hydroxyundecanoic acid lactone; 4-Undecanolide; 5-Heptyldihydro-2(3H)-furanone; NSC 406421; NSC 46118; NSC 76413; Neutralizing agent 350120-1; Peach lactone; Peche Pure; Persicol; γ-(n-Heptyl)-γ-butyrolactone; γ-Heptyl-γ-butyrolactone; γ-Heptylbutyrolactone; γ-Undecalactone; γ-Undecanolactone; γ-Undecanolide; γ-n-Heptylbutyrolactone	

Current regulation: /

Clinical data: /

Additional information:

It is a "top 100" substance (IFRA, pers. comm. 2010)

VANILLIN	
CAS # 121-33-5	
EC # 204-465-2	

4-Hydroxy-3-methoxybenzaldehyde	
2-Methoxy-4-formylphenol; 3-Methoxy-4-hydroxybenzaldehyde; 4-Formyl-2-methoxyphenol; 4-Hydroxy-5-methoxybenzaldehyde; 4-Hydroxy-m-anisaldehyde; H 0264; Lioxin; NSC 15351; NSC 403658; NSC 48383; Rhovanil; Vanillaldehyde; Vanillic aldehyde; Vanillum; m-Methoxy-p-hydroxybenzaldehyde; p-Hydroxy-m-methoxybenzaldehyde; p-Vanillin	

Current regulation: /

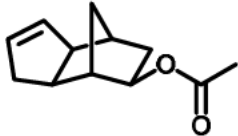
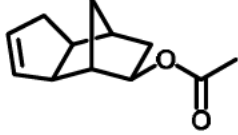
Clinical data:

In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to vanillin was observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reaction to vanillin 10 % pet. (22). The IVDK 2010 study, n=10, i.e., 0.19% (95% CI: 0.07 – 0.32%; percentages standardised for age and sex) of 4377 patients PTed reacted to the compound, tested 10% pet. (7). In n=102 patients with a positive reaction to MPR, 19 compounds of this natural mixture were tested, among these, vanillin, to which none reacted positively (208). In 21 patients with contact allergy to propolis, 2 also reacted to vanillin (10% pet.) (209).

A 13-year-old girl with recurrent (peri-)cheilitis after application of a vanilla lip salve tested strongly positive to this salve (as is), "Vanilla 10% pet." (unclear, whether natural extract or vanillin) and MPR (210). Trattner/David identified 1 / 641 consecutive patients with positive reaction to vanillin (31).

Additional information:

Naturally occurring in the fruit of *Vanilla planifolia* after a fermentation process, in styrax, clove oil, potatoes, wood, including Myroxylon pereirae resin, and other material (53). Nowadays, vanillin is synthesised from eugenol, guajakol and lignin residues from paper production, however, not fully achieving the subtle scent and taste of the natural material (53). It is a "top 100" substance and classified as R43 (IFRA, pers. comm. 2010).

VERDYL ACETATE	
CAS # 2500-83-6/ 5413-60-5	
EC # 219-700-4 / 226-501-6	
3a,4,5,6,7,7a-Hexahydro-4,7-methanoinden-6-yl acetat (2500-83-6)	 2500-83-6
3a,4,5,6,7,7a-Hexahydro-4,7-methano-1H-inden-5-yl acetat (5413-60-5)	 5413-60-5
2500-83-6: 4,7-Methano-1H-inden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methanoinden-5-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; NSC 142428; NSC 94573	
5413-60-5: 4,7-Methano-1H-inden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 4,7-Methano-3a,4,5,6,7,7a-hexahydroinden-6-yl acetate; 8-Acetoxytricyclo[5.2.1.0 ^{2,6}]dec-3-ene; Greenyl acetate;	

Herbaflorat; Jasmacyclene; NSC 6598	
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Current regulation: /

Clinical data: /

Additional information:

In CosIng, both above CAS numbers are listed under "verdyl acetate" (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41289>, last accessed 2010-07-19).

In the CAS, there are 2 separate entries; moreover, there are 2 separate RIFM reviews:

- # 2500-83-6: Other names: Tricyclo[5.2.1.0^{2,6}]dec-4-en-8-yl acetate (REACH, EINECS, INCI Name according to CAS); 3a,4,5,6,7,7a-Hexahydro-4,7-methanoinden-6-yl Acetate; Tricyclodecen-4-yl 8-Acetate. It is a "top 100" substance (IFRA, pers. comm. 2010). A RIFM review is available, stating that "no data is available" regarding the skin sensitising properties of the substance (211).
- # 5413-60-5: Other names: 3a,4,5,6,7,7a-hexahydro-4,7-methanoinden-6-yl acetate (REACH, EINECS, INCI Name according to CAS), 4,7-Methano-3a,4,5,6,7,7a-hexahydroinden-6-yl acetate; 4,7-Methanoinden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate; 8-Acetoxytricyclo[5.2.1.0^{2,6}]dec-3-ene; Tricyclodecenyl acetate; Greenyl acetate; Herbaflorat; Jasmacyclene; NSC 6598; Verdyl acetate. It is a "top 100" substance (IFRA, pers. comm. 2010). A RIFM review is available (212), citing 2 negative human maximisation tests and 1 negative HRIPT.

Natural extracts / essential oils

Natural raw materials in terms of extracts are used in the fragrance and flavour industry for various reasons. Most importantly, several naturally occurring mixtures have a very complex composition and sensory nature which cannot (fully) be achieved by synthetic material. Moreover, several compounds cannot be synthesised at a competitive price, and the demand for perfumes based on natural materials is considerable (34).

The three main methods used to concentrate plant fragrance substances as essential oils comprise steam distillation, mechanical processes from the epicarp of Citrus fruits ("pressing") and dry distillation. An Essential oil is „obtained by steam distillation with addition of water in the still (hydrodistillation) or without addition of water in the still (directly by steam)"(213). Essential oil of fruit juice is „obtained by from a fruit juice during its concentration or during UHT (flash pasteurization) treatment" (213). Cold pressed essential oil is „obtained by mechanical processes from the epicarp of the fruit of a Citrus, at ambient temperature"(213). Citrus peel oils, apart from distilled Citrus oils, are produced with various methods (214). The oil consists of a high volume of volatile terpenes, mostly monoterpenes but also contains small amounts of non-volatile compounds such as dyes, waxes and furocoumarines.

The method of solvent extraction is generally applied in the separation of heat-labile materials or if an essential oil can only be obtained in very low yield, e.g. from blossoms. It is also used if the non-volatile components are desired for their fixative properties, e.g. in the preparation of resinoids from exudates. The most important extracts are termed: (i) concrete: an extract „obtained from a fresh plant natural raw material by extraction with a solvent"¹⁸, containing not only volatile, but also a large proportion of non-volatile substances such as waxes; and (ii) absolute: „product, obtained by extraction with ethanol from a concrete, a floral pomade, a resinoid or a supercritical fluid extract. The ethanolic solution is generally cooled down and filtered in order to eliminate the «waxes»; the ethanol is then eliminated by distillation"¹⁹. Resinoids, used for their fixative properties, are „obtained from a dry plant natural raw material by extraction with a solvent"²⁰. The products are usually highly viscous and thus might sometimes be diluted, e.g. with phthalates or benzyl benzoate. Oleoresins are extracts „of spice or aromatic herb" by „treating a natural raw material with a solvent, then, after filtration if necessary, the solvent is eliminated"²¹.

Regarding clinical data in terms of contact allergy to fragrance ingredients, the main focus of case report or clinical studies regarding essential oils and natural extracts, respectively, is on general dermatological patients with complaints related to use of cosmetics etc. However, series of cases with occupational exposure to essential oils with occupational allergic contact dermatitis have also been reported (e.g., masseurs,

¹⁸ ISO/DIS 9235

¹⁹ ISO/DIS 9235

²⁰ ISO/DIS 9235

²¹ ISO/DIS 9235

physiotherapists (215, 216), aromatherapists (217-221), beauticians doing massages (222); for further details, e.g., PT results with various essential oils, see original case reports. "Current Regulation" refers to the EU Cosmetics Directive only.

Catalogue of natural extracts / essential oils evaluated

ACORUS CALAMUS ROOT OIL

CAS 84775-39-3; EC 283-869-0

Calamus Oil; "Sweet Flag Oil"

(*Acorus calamus*, ext. = INCI name)

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to "calamus" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to "calamus" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Acorus calamus* L. (sweet flag calamus). *Acorus Calamus Root Oil* is an essential oil obtained from the rhizomes of the calamus, *Acorus calamus* L., Araceae. It contains beta-asarone (up to 96%, depending on ploidy, and with this, origin (34)), calamene (about 4%), calamol (about 3%) alpha-asarone (about 1%), camphene (about 1%) and some beta-pinene and asaronaldehyde (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41330>, last accessed 2010-01-29). Use is restricted due to potential toxicity of beta-asarone (34).

CANANGA ODORATA and Ylang-ylang oil

Ylang-ylang and cananga oils are essential oils that are obtained from two subspecies of the cananga tree (34). In the INCI nomenclature, both are not differentiated.

CANANGA ODORATA FLOWER EXTRACT

CAS 83863-30-3; EC 281-092-1
(ylang-ylang, ext.) INCI name:
CANANGA ODORATA EXTRACT

CANANGA ODORATA FLOWER OIL

CAS 8006-81-3, 68606-83-7; EC / (oils,
ylang-ylang) INCI name: CANANGA
ODORATA OIL

Current regulation: ...

Clinical data:

Ylang-ylang oil

ISO 4720:2009 nomenclature: *Cananga odorata* (Lam.) Hook. f. et Thomson *forma*

genuina)

In the Larsen 2002 c study, “synthetic ylang-ylang oil” caused 6.4% positive reactions in 218 patients with known contact allergy to fragrance ingredients (1). In a Japanese study, M. Sugawara et al. noted a significant decline of the proportion of patients reacting positively to “ylang-ylang oil 5% pet.” from 1971 to 1989, the overall number in patients with cosmetic dermatitis amounting to 176 of 1438 (12.2%, 95% CI: 10.6 – 14.0%) (223). In the Frosch 2002 b study, two fractions of Ylang-Ylang oil (I and II) were separately tested, each at 10% pet. Fraction I yielded 2.6%, fraction II 2.5% positive test reactions (no data on concomitant reactivity given) (17). The deGroot 2000 study, with 1825 consecutively tested patients, found 18 positive PT reactions to “ylang-ylang oil”, tested at 4% in pet. (12). The Sugiura 2000 study with 1483 patients with suspected cosmetic dermatitis observed 0.8% positive PT reactions with ylang-ylang oil (5% pet.) (14). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with ylang-ylang oil (2% pet.) 13.4% positive reactions (9). The Belsito 2006 study (20) yielded 0.6% positive reactions to ylang-ylang oil. The subsequent NACDG 2009 study identified 1.5% positive reactions in 4434 patients PTed with 2% “ylang-ylang oil” (21). The IVDK 2010c study found 2.5% positive reactions in 3175 consecutively tested patients, and 3.9% in 2155 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=12 reacted positively to ylang-ylang oil and 3 to “cananga oil” (48).

Cananga oil

ISO 4720:2009 nomenclature: *Cananga odorata* (Lam.) Hook. f. et Thomson *forma macrophylla*. For Oil of cananga (*Cananga odorata* (Lam.) Hook. f. et Thomson, *forma macrophylla*) an ISO standard exists: ISO 3523:2002. Cananga oil is produced by steam distillation of the flowers of *Cananga odorata* (DC.) Hook f. et Thomson subsp. *macrophylla* (*Annonaceae*). The composition resembles that of “ylang-ylang III”, but with a higher content of caryophyllene (30-40%). Cananga oil originates almost exclusively in Java; annual production about 50 t. The oil is used mainly in perfuming soaps where it is more stable than ylang-ylang oils due to its lower ester content (34).

Sugiura et al. (2000) found 1.1% positive reactions to “cananga oil”, tested 5% pet. (14). Cananga oil (2% pet.) mentioned in the same Portuguese study already cited (9) yielded 10.4% positive reactions. In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to cananga odorata oil tested at 2% concentration (13).

Studies with both oils

The Goossens 1997 study found 3 of 111 patients positive to “ylang-ylang oil 5% pet.”, and 4 to “cananga oil 15% pet.” – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found 1 positive reaction in 200 patients to “cananga” and 4 to “ylang-ylang” essential oil, both tested 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=10 (11.6%) positive reactions to “cananga” and n=8 (9.3%) to “ylang-ylang” essential oils, each tested at 2% pet. (27). Nakayama et al. found 1974 (after (29)) 11 “strong positive” and 15 “weak positive” reactions to “Cananga oil” and 9 and 16, resp., to “Ylang-ylang oil” (unknown test concentration) in 183 patients.

A number of case reports highlight the possibility of occupational contact and sensitisation, e.g. (222, 224).

Additional information:

Ylang-ylang oil

The composition of this essential oil is defined by a standard: ISO 3063:2004. Ylang-ylang oils are obtained by steam distillation of freshly picked blossoms of *Cananga odorata* (DC.) Hook f. et Thomson subsp. *genuina* (*Annonaceae*). The oil is produced mainly in Madagascar and the Comoro islands. Four fractions are collected at progressively longer distillation times and are known as "extra", "I", "II" and "III". The composition of the various oil fractions depends on the duration of distillation. The first fraction has the highest content of strongly odiferous constituents such as p-cresyl methyl ether (5-16%), methyl benzoate (4-9%), (-)-linalool (7-24%), benzyl acetate (5.5-17.5%), and geranyl acetate (2.5-14%). The other fractions contain increasing amounts of sesquiterpene hydrocarbons such as caryophyllene, germacrene-D, and (E,E)-alpha-farnesene (> 70% in "ylang-ylang III"). Components such as p-cresol, eugenol and isoeugenol are important for odour, although they are present only in low concentration (34). According to (30) the maximum observed concentration in ylang-ylang I and II are (in %): germacrene-D (28); (E,E)-alpha-farnesene (21); caryophyllene (17); linalool (I: 19.0; II: 9.5); benzyl benzoate (8.0); farnesol (4.0); benzyl salicylate (4.0); (E,E)-farnesyl acetate (3.5); geraniol (2.5); isoeugenol (0.8); benzyl alcohol (0.5); eugenol (0.5); p-cresyl methyl ether (I: 5.0; II 3.5); methyl benzoate (I: 5.5; II: 3.5); benzyl acetate (I: 10.0; II: 5.0); geranyl acetate (I: 15.0; II: 12.0).

CEDRUS ATLANTICA BARK OIL

CAS 92201-55-3; EC 295-985-9
(*Cedrus atlantica*, ext. = INCI) /
8000-27-9; EC / (Oils,
cedarwood) INCI name: CEDRUS
ATLANTICA OIL

Cedarwood oil

Current regulation: /

Clinical data:

In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=5 (0.7%) positive reactions to cedarwood oil 10% pet. (22). (The exact origin of "cedarwood oil" in this study is not clear.) The IVDK 2010 c study identified 0.8% positive reactions in 6223 patients tested in the context of a special series with a cedarwood oil tagged with CAS # 8000-27-9 (30).

Additional information:

Cedrus Atlantica Bark Oil is the volatile oil obtained from the bark of *Cedrus atlantica*, *Pinaceae*

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55309>, last accessed 2010-01-05). The main odiferous component is alpha-atlantone [32207-08-2] (39)

Nomenclature also used: *Cedrus atlantica* wood oil (*Cedrus atlantica* (Endl.) G.Manetti ex Carrière)²²

See also *Juniperus virginiana*.

²² ISO 4720:2009 nomenclature

CEDRUS DEODARA WOOD OILCAS 91771-47-0; EC 294-939-5 (*Cedrus deodara*, ext.)*Cedarwood oil*

Current regulation: /

Clinical data:

The Rudzki 1976 study found 3 positive reactions in 200 patients to "cedarwood" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "Himalayan cedarwood" essential oil 2% pet. (27). (The labelling in the latter report points to *Cedrus deodara* as source of "cedarwood oil" in these 2 Polish studies.)

Additional information:

Cedrus Deodara Wood Oil, Himalayan cedarwood oil (*Cedrus deodara* (Roxb. ex D.Don) G. Don)²³, is the volatile oil obtained by steam distillation of the stumps of the Deodar Cedar, *Cedrus deodara*, *Pinaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55311>, last accessed 2010-01-29).

Several other conifer species are called cedars, and the corresponding oils vary considerably in composition. These include Cedar leaf oil (*Thuja* oil) produced by steam distillation of fresh leaves and branch ends of *Thuja occidentalis* L. (*Cupressaceae*) from North America, containing a minimum of 60% thujone [8007-20-3] [90131-58-1] (34). Texas cedarwood oil is produced by steam distillation of chopped wood of *Juniperus mexicana* Schiede (*Cupressaceae*), containing alpha-cedrene (15-25%), thujopsene (25-35%), cedrol 20% minimum [8000-27-9] [91722-61-1] (34). Chinese cedarwood oil is similar to Texas cedarwood oil, obtained by steam distillation of *Cupressus funebris* Endl., *Cupressaceae* (*Chamaecyparis funebis* (Endl.) France), which is a weeping cypress [8000-27-9] [85085-29-6] (34).

CINNAMOMUM CASSIA LEAF OIL

94961-46-6 [invalid] / 8007-80-5; EC / (Oils, cassia) INCI name: CINNAMONUM CASSIA OIL

*Cassia Oil; Cassia leaf Oil; Cinnamon Oil Chinense***CINNAMOMUM ZEYLANICUM BARK OIL**CAS 84649-98-9; EC 284-635-0 (*Cinnamomum zeylanicum*, ext. = INCI)*Cimmamon Bark Oil Ceylon; Cinnamon Oil Ceylon*

Current regulation: /

Clinical data:

The Rudzki 1976 study found 2 positive reactions in 200 patients to "cassia" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=24

²³ ISO 4720:2009 nomenclature

(27.9%) positive reactions to "cassia" essential oil 2% pet. (27).

A 32 year old Spanish physiotherapist developed vesicular hand dermatitis after using a "balsam from ash extract" cream. PTing revealed positive reactions to this cream, the FM I, eugenol, and 2 components of the cream: "cinnamon oil" (0.5% pet.) and clove oil (1% pet.) (225).

Additional information:

ISO 4720:2009 nomenclature: *Cinnamomum tsumu* Helms, syn. *Cinnamomum cassia* auct. and *Cinnamomum zeylanicum* Blume syn. *Cinnamomum verum* J. Presl, respectively. Cassia oil (Chinese cinnamon oil) is obtained by steam distillation of the leaves, twigs, and bark of *Cinnamomum aromaticum* Nees (*C. cassia* Blume, *Lauraceae*). In contrast to cinnamon bark oil (see below), cassia oil contains a considerable amount of 2-methoxycinnamal (3-15%), in addition to its main constituent, cinnamal (70-88%). Cassia oil is predominantly used in flavouring soft drinks, with an annual production of a few hundred tons (34). For Oil of cassia, Chinese type (*Cinnamomum aromaticum* Nees, syn. *Cinnamomum cassia* Nees ex Blume) an ISO standard exists: ISO 3216:1997

Cinnamomum Zeylanicum Bark Oil is the volatile oil expressed from the bark of the Ceylon Cinnamon, *Cinnamomum zeylanicum*, *Lauraceae*. It contains mainly cinnamaldehyde (34), e.g. 50-60%, and lesser quantities of eugenol (4-8%), phellandrene

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75370>, last accessed 2009-11-16). For Oil of cinnamon leaf, Sri Lanka type (*Cinnamomum zeylanicum* Blume) an ISO standard exists: ISO 3524:2003

Cinnamomum Cassia Leaf Oil is the volatile oil obtained by steam distillation from the leaves and twigs of the Chinese Cinnamon, *Cinnamomum cassia* (L.), *Lauraceae*. It contains 80% eugenol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75368>, last accessed 2009-11-16). The cinnamon leaf oil produced by steam distillation of the leaves of *Cinnamomum zeylanicum* Blume (*C. verum* J.S. Presl) similarly has a content of 70-83% eugenol (34).

Considering the content of well-known allergenic compounds, the essential oil is considered an Established contact allergen in humans,

CITRUS AURANTIUM AMARA FLOWER OIL

CAS 8016-38-4, 68916-04-1; EC / (Oils, neroli) /

Neroli oil

CITRUS AURANTIUM AMARA PEEL OIL EXPRESSED

72968-50-4; EC 277-143-2 (Orange, sour, ext.)

"Bitter Orange Oil"

INCI names: CITRUS AURANTIUM AMARA ...

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "neroli oil" (2% pet.) 6.6% positive reactions (9). The Rudzki 1976 study found 3 positive reactions in 200 patients to "bitter orange" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%)

positive reactions to "bitter orange" essential oil 2% pet. (27). The IVDK 2010 c study identified 0.7% positive reactions in 6220 patients tested in the context of a special series (30)

Additional information:

ISO 4720:2009 nomenclature: *Citrus aurantium* L., syn. *Citrus amara* Link, syn. *Citrus bigaradia* Loisel, syn. *Citrus vulgaris* Risso. For Oil of neroli (*Citrus aurantium* L. spp. *aurantium*, syn. *Citrus aurantium* L. spp. *amara* var. *pumilia*) an ISO standard exists: ISO 3517:2002. Citrus Aurantium Peel Oil Expressed is an essential oil expressed from the fresh epicarps of the Sour Orange, *Citrus aurantium*, Rutaceae. It contains D-limonene (about 90%), citral, decanaldehyde, methyl anthranilate, linalool, terpineol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41394>, last accessed 2010-01-29). The aldehyde content is lower and the ester content (e.g., linalyl and geranyl acetate) is higher than in sweet orange oil (34). It is predominantly used for flavouring alcoholic beverages. According to (30) the maximum observed concentration in neroli oil are (in %): linalool (44); limonene (18); β -pinene (17); linalyl acetate (15); *trans*- β -ocimene (8); geranyl acetate (5); *trans*-nerolidol (5); (*E,E*)-farnesol (4); myrcene (4); farnesol (4,0); geraniol (3,5); citral (0,3) (30).

CITRUS AURANTIUM AMARA LEAF OIL

72968-50-4; EC 277-143-2 (Orange, sour, ext.)

Petitgrain oil Paraguay / ... bigarade

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "Petitgrain bigarade" and "Petitgrain Paraguay" essential oil each, both tested at 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to "Petitgrain bigarade" and n=4 (4.6%) to "Petitgrain Paraguay" essential oil each, both tested at 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Citrus sinensis* L. Pers. X *Citrus aurantium* L. ssp. *amara* var. *pumilia*. Petitgrain oils in general are steam distilled from the leaves of citrus trees. Citrus Aurantium Leaf Oil is an essential oil obtained from the leaves of the Sour Orange, *Citrus aurantium*, Rutaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41392>, last accessed 2010-02-10). Petitgrain oil Paraguay is obtained from an acclimatised variety of the bitter orange tree. Main constituents are linalool (15-30%) and linalyl acetate (40-60%). A number of trace constituents contribute essentially to the odour (34). Petitgrain oil bigarade is derived from the same species of tree grown in France, Italy, Spain and North Africa (34). For Oil of bitter orange petitgrain, cultivated (*Citrus aurantium* L.) an ISO standard exists: ISO 8901:2003.

Considering the content of well-known allergenic compounds, the essential oil is regarded as an established contact allergen in humans

CITRUS BERGAMIA PEEL OIL EXPRESSED

CAS 89957-91-5, 8007-75-8; EC

	289-612-9 (<i>Bergamot, ext.</i>)
<i>Bergamot Oil, Bergamot Orange Oil</i>	INCI: CITRUS AURANTIUM BERGAMIA EXTRACT

Current regulation: /

Clinical data:

The Rudzki 1976 study found 3 positive reactions in 200 patients to "Bergamot" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found no positive reaction to "Bergamot" essential oil 2% pet. (27). In 63 patients positive to the FM I, 2 had a positive PT reaction to bergamot oil, 2% pet., in the Santucci 1987 study (28). A case report from Zacher and Ippen describes 2 patients with allergic contact dermatitis due to bergamot oil (191), one a worker in a perfume factory, the other sensitised by non-occupational use of cosmetics.

Additional information:

ISO 4720:2009 nomenclature: *Citrus bergamia* (Risso et Poit.), syn. *Citrus aurantium* L. subsp. *bergamia* (Wight et Arnott) Engler. Citrus Bergamia Peel Oil Expressed is an essential oil expressed from the epicarps of the Bergamot, *Citrus bergamia* risso, Rutaceae. It contains 35-45% L-linalyl acetate, about 6% linalool, D-limonene, DL-limonene and bergaptene (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=se.arch.details&id=41398>, last accessed 2009-11-27). According to Surburg/Panten: linalyl acetate 22-36%, linalool 3-15%, geranial 0.25-0.5%, citral 1%, with a relatively low terpene content of 25-50% (34, 39). Bergaptene content by HPLC is 0.18-0.38% (34). Annual production from Italy, Brazil, Spain and Ivory Coast is 100 to 150 t. For Oil of bergamot [*Citrus aurantium* L. subsp. *bergamia* (Wight et Arnott) Engler], Italian type an ISO standard exists: ISO 3520:1998.

CITRUS LIMONUM PEEL OIL EXPRESSED

CAS 84929-31-7, 8008-56-8; EC 284-515-8 (*Lemon, ext.*)

Lemon oil

INCI names: CITRUS MEDICA
LIMONUM ...

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "lemon oil" (2% pet.) 4.5% positive reactions (9). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=2 (0.3%) positive reactions to "lemon oil" 2% pet. (22).

The Rudzki 1976 study found 1 positive reaction in 200 patients to "lemon" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to "lemon" essential oil 2% pet. (27). The IVDK 2010 c study identified 0.3% positive reactions in 6467 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Citrus limon* (L.) Burm. f. According to (30) the maximum observed concentration in lemon oil are (in %): limonene (80); β -pinene (16.5); γ -terpinene (12); citral (3.0); geranial (2.0); neral (1.2); β -bisabolene (0.9); geranyl

acetate (0.7); neryl acetate (0.6); linalool (0,3); geraniol (0,2) (30). An ISO standard exists for Oil of lemon [*Citrus limon* (L.) Burm. f.], obtained by expression: ISO 855:2003. The composition of lemon oil depends on the variety of lemon and the country of origin, see table from (34).

Table 3. Specifications for qualities of lemon oils of different origins

Parameter	Type	Mediterranean		Equatorial	
	American Origin				
	Coast	Desert	Italy	Spain	Ivory coast, Brazil
d_{20}^{20}	0.851–0.857	0.849–0.854	0.850–0.858	0.849–0.858	0.845–0.854
n_D^{20}	1.4370–1.4760	1.4370–1.4760	1.4370–1.4760	1.4370–1.4760	1.4370–1.4790
α_D^{20}	+57° to +65°6'	+67° to +78°	+57° to +66°	+57° to +66°	+57° to +70°
Composition by GC [area %]					
β -Pinene	9–14	10–13	10–16.5	10–16.5	7–16
Limonene	63–70	70–80	60–68	60–70	59–75
γ -Terpinene	8.3–9.5	6.5–8	8–12	8–12.8–12	6–12
Neral	0.6–0.9	0.3–0.6	0.6–1.2	0.4–1	0.2–1.2
Geraniol	1.0–2	0.5–0.9	0.8–2	0.6–2	0.5–2
Evaporation residue [weight %]					
	1.75–3.9		1.5–3.9	1.5–3.9	1.5–4
Carbonyl value					
	8–14	6.25–12	11–17	11–17	6–17
CD value	min. 0.2	min. 0.2	0.45–0.9	0.4–0.9	0.2–0.96

CITRUS PARADISI PEEL OIL

Grapefruit oil, expressed

CAS 8016-20-4 ; EC /

INCI: CITRUS GRANDIS OIL

Current regulation: II/358 R1

Clinical data: /

Additional information:

Citrus Paradisi Peel Oil is the volatile oil expressed from the peel of the Grapefruit, Citrus paradisi, Rutaceae
http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=55434

It is a "top 200" substance and classified as R43 (IFRA, pers. comm.2010)

CITRUS SINENSIS (syn.: AURANTIUM DULCIS) CAS 97766-30-8, 8008-57-9, EC
 PEEL OIL EXPRESSED 307-891-8 (Orange, sweet,

(Sweet) Orange oil

Valencia, ext. = INCI) / 8028-48-6; EC 232-433-8 (Orange, sweet, ext.)

INCI names: CITRUS AURANTIUM DULCIS ...

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "orange oil" (2% pet.) 4.5% positive reactions (9). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reactions to orange oil 2% pet. (22). The Rudzki 1976 study found 1 positive reaction in 200 patients to "sweet orange" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "sweet orange" essential oil 2% pet. (27). In the Frosch 1995 dose-finding pilot study, neither positive nor irritant reaction to 1% and 5% "orange oil Bras." in pet., tested in 205 consecutive patients in Dortmund and Göttingen, were observed (15). The IVDK 2010 c study identified 0.2% positive reactions in 6246 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Citrus sinensis* (L.) Osbeck. For Oil of sweet orange (*Citrus sinensis* (L.) Osbeck), CAS 8008-57-9, obtained by mechanical treatment, an ISO norm exists: ISO 3140:2005. The oils have a high terpene hydrocarbon content (> 90%, mainly (+)-limonene. Important for aroma are aldehydes, mainly decanal and citral, and aliphatic and terpenoid esters. The sesquiterpene aldehydes alpha-sinensal [17909-77-2] and beta-sinensal [6066-88-8] contribute particularly to the special sweet aroma (34). According to (30) the maximum observed concentration in sweet orange oil are (in %): *limonene* (95.0); *linalool* (0.7); *n*-decanal (0.7); *citral* (0.3); alpha-sinensal (0.05); beta-sinensal (0.06) (30). Worldwide production is more than 30000 tons / year. Main uses comprise the flavouring of beverages and confectioneries and perfuming E.d.C, soaps and household products.

For the latter uses relevant here, both "Orange peel oil, sweet (*Citrus sinensis* (L.) Osbeck) (8008-57-9)", "Orange peel, sweet, extract (*Citrus sinensis* L. Osbeck) (8028-48-6)" and "Orange, sweet, Valencia, ext. (97766-30-8)" are among the top 100 used fragrance materials and classified as R43 (IFRA, pers. comm. 2010).

ORANGE OIL TERPENES (CAS # 68647-72-3) are a "top 100 mixture of substances and classified as R43 (IFRA, pers. comm.2010). Other names: ORANGE, SWEET, TERPENES (REACH); Terpenes and Terpenoids, sweet orange-oil (REACH). The CAS entry refers to a group of substances "Terpenes and Terpenoids, sweet orange-oil" (REACH).

CITRUS TANGERINA ...

Oil of tangerine

CAS 223748-44-5; EC /

[no info in CAS database]

Current regulation: /

Clinical data:

In a 17 year old girl, the perfume used for 3 months caused ACD due to the ingredient "oil of tangerine", with a strong positive PT reaction (to 2% or 10% in pet.; 50 controls

negative) (226).

Additional information:

Citrus Tangerina Peel Oil is the volatile oil expressed from the peel of the ripe fruit the Tangerine, *Citrus Tangerina*, *Rutaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=55441>, last accessed 2010-01-29); (*Citrus tangerina* Tanaka).

CORIANDRUM SATIVUM HERB OIL

CAS 84775-50-8; EC 283-880-0
(Coriander, ext.)

Coriander oil

INCI: CORIANDRUM SATIVUM
EXTRACT

Current regulation: /

Clinical data:

The Rudzki 1976 study found 2 positive reactions in 200 patients to "coriander" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "coriander" essential oil 2% pet. (27).

Additional information:

Coriander Sativum Herb Oil is an essential oil obtained from the herbs of the Coriander, *Coriandrum sativum* L., *Umbelliferae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39388>, last accessed 2010-01-29). The main component of coriander oil is linalool (by GC: 65-78%) and mono- and polyunsaturated fatty aldehydes contributing to the particular aroma. In contrast to the seed oil, coriander leaf oil contains these aldehydes as main constituents, e.g. 2-deccanal and 2-dodecanal (34). For Oil of coriander fruits (*Coriandrum sativum* L.) an ISO standard exists: ISO 3516:1997.

CYMBOPOGON OILS

Cymbopogon oils are produced from several aromatic grasses that belong to the genus *Cymbopogon* Speng. (*Poaceae*). The oils are obtained by steam distillation of the aerial parts of the plants (34).

The composition of the essential oil derived from *Cymbopogon flexuosus* (Nees ex Steudel) J.F. Watson is defined by a standard: ISO 4718:2004, as is the oil derived from *Cymbopogon citratus*: 3217:1974.

CYMBOPOGON CITRATUS LEAF OIL

Cymbopogon citratus (DC.) Stapf.²⁴

CAS 89998-14-1; EC 289-752-0
(*Cymbopogon citratus*, ext. =
INCI)

²⁴ ISO 4720:2009 nomenclature

Lemon Grass Oil; Indian Verbena Oil; Indian Melissa Oil

CYMBOPOGON SCHOENANTHUS OIL

Cymbopogon flexuosus (Nees ex Steudel) J.F. Watson²⁵

CAS 8007-02-1; EC 289-754-1 (oils, lemongrass) / 89998-16-3; EC 289-752-0 (Cymbopogon Schoenanthus, ext. = INCI)

Lemon Grass Oil

Current regulation: /

Clinical data:

The Frosch 2002 b study on 1606 consecutive patients reported 1.6% positive reactions to "lemongrass oil (East India), CAS 8007-02-1", PTed at 2% pet. (17). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 3 positive reactions to lemongrass oil were observed (3). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=6 (0.8%) positive reactions to lemongrass oil 2% pet. (22). The IVDK 2010 c study identified 0.6% positive reactions in 2435 consecutively tested patients and 2.3% positive reactions in 8445 patients tested in the context of a special series (30).

Additional information:

Cymbopogon Citratus Leaf Oil is an essential oil obtained from the leaves of the Lemon Grass, *Cymbopogon citratus* (DC., ex Nees), *Poaceae*. It contains citral (75-85%), methylheptenone, citronellal, geraniol, limonene (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39457>, last accessed 2009-11-12). According to Surburg/Panten, by GC: neral (31-40%), geranial (40-50%) (34).

Indian lemongrass oil is obtained by the so-called Indian variety of lemongrass, *Cymbopogon flexuosus* (Nees ex Steud.) Stapf. Content by GC: 25-35% neral, 35-47% geranial (34).

Cymbopogon Schoenanthus Oil is the volatile oil obtained by the steam distillation of fresh Lemon Grass, *Cymbopogon schoenanthus* (L.), *Poaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=75419>, last accessed 2009-11-12). According to (30) the maximum observed concentration in lemongrass oil are (in %): citral (85.0); geraniol (7.0); limonene (4.0); geranyl acetate (2.2); caryophyllene (1.6); trans-isocitral (1.4); 6-methyl 5-hepten-2-one (1.3); caryophyllene oxide (1.2); 4-nonanone (1); citronellol (0.8); eugenol (0.3); linalool (0.2) (also according to (227))

In a LLNA study by RIFM, the lemongrass oil as used was reported to contain 68.8% citral, 6.7% limonene, 6.1% geraniol, 2.2% geranyl acetate, 1.6% caryophyllene, 1.4% trans-isocitral, 1.3% 6-methyl 5-hepten-2-one, 1.2% caryophyllene oxide and 1% 4-nonanone, according to analyses of the supplier. The EC3 value was calculated to be 6.5% (227).

CYMBOPOGON MARTINI HERB EXTRACT

CAS 84649-81-0; EC 283-461-2 (Cymbopogon Martini, ext)

²⁵ ISO 4720:2009 nomenclature

INCI: CYMBOPOGON MARTINI OIL*Palmarosa oil*

Current regulation: /

Clinical data: /

Additional information:

ISO 4720:2009 nomenclature: *Cymbopogon martini* (Roxb.) Will. Watson var. *motia* and var. *sofia*. Cymbopogon Martini Herb Extract is an extract obtained from the herbs of the plant, *Cymbopogon martini*, Gramineae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39460>, last accessed 2009-11-24), namely, by steam distillation of wild or cultivated *Cymbopogon martini* (Roxb.) J.F. Wats., collected when in blossom (34). The main constituent is geraniol (72-94%) (34).

In a LLNA study by RIFM, the palmarosa oil as used was reported to contain 79.4% geraniol, 9.4% geranyl acetate and 1.9% caryophyllene, according to analyses of the supplier. The EC3 value was calculated to be 9.6% (227).

CYMBOPOGON NARDUS HERB OIL

CAS 89998-15-2; EC 289-753-6 (*Cymbopogon nardus*, ext. = INCI)

Citronella Oil (Sri Lanka)

CYMBOPOGON WINTERIANUS HERB OIL

CAS 91771-61-8; EC 294-954-7 (*Cymbopogon Winterianus*, ext. = INCI)

Citronella Oil (Java)

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 5 positive reactions in 200 patients to "citronella" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=1 (1.1%) positive reactions to "citronella" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Cymbopogon nardus* (L.) W. Watson var. *lenabatu* Stapf. and *Cymbopogon winterianus* Jowitt, respectively. Cymbopogon Nardus Herb Oil is an essential oil obtained from the herbs of the plant, *Cymbopogon* (syn: *Andropogon*) *nardus* (L.), Gramineae. The Ceylon citronella oil contains geraniol (about 60%), citronellal (about 15%), camphene, limonene, linalool, borneol. According to Surburg/Panten, the Sri Lankan oil contains citronellal (3-6%), borneol (4-7%), citronellol (3-8.5%), geraniol 15-23% and methyl isoeugenol (7.11%) (34).

The Java citronella oil contains 25-50% citronellal, 25-45% geraniol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.h.details&id=39469>, last accessed 2009-11-24). Cymbopogon Winterianus Herb Oil as a synonym for Java citronella oil is obtained from the herbs of the plant, *Cymbopogon winterianus*, Gramineae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=sea>

[rch.details&id=39472](#), last accessed 2009-11-24). This oil, produced in Taiwan and Java, contains citronellal (31-40%), geraniol (20-25%), citronellol (8.5-14%), geranyl acetate (2.5-5.5%), citronellyl acetate (2-4%) and many minor components. Annual worldwide production is currently at around 1000 t (34). For Oil of citronella, Sri Lankan type (*Cymbopogon nardus* (L.) W. Watson var. *lenabatu* Stapf.) an ISO standard exists: ISO 3849:2003, for Oil of citronella, Java type the ISO 3848:2001.

In a LLNA study by RIFM, the citronella oil as used was reported to contain 36.6% citronellal, 20.6% geraniol, 4.1% limonene, 3.7% geranyl acetate, 3.0% citronellyl acetate, 2.6% elemol, 2.2% beta-bourbonene, 1.9% delta-cadiene, 1.6% isopugenol I, 1.4% germacrene D and eugenol and linalol at < 1%, according to analyses of the supplier. The EC3 value was calculated as > 50 % (227).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

EUCALYPTUS SPP. LEAF OIL

CAS 92502-70-0; EC 296-357-7
(*Eucalyptus*, ext. = INCI)

Eucalyptus Oil

CAS 8000-48-4; EC / (Oils,
eucalyptus) INCI: *EUCALYPTUS*
GLOBULUS OIL

Current regulation: /

Clinical data:

In a study with 218 fragrance sensitive patients, 1.8% reacted positively to 10% eucalyptus oil (pet.) (1). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 1 positive reaction to "eucalyptus oil" was observed (3). In the Wöhrl 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=4 (0.6%) positive reactions to eucalyptus oil 2% pet. (22). The Rudzki 1976 study found 3 positive reactions in 200 patients to "eucalyptus" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=1 (1.1%) positive reactions to "Eucalyptus" essential oil 2% pet. (27). The IVDK 2010 c study identified 0.2% positive reactions in 6680 patients tested in the context of a special series (30).

In a professional athlete, the use of an "analgesic and anti-inflammatory cream" over 2 years lead to ACD, which was attributed to eucalyptol (eucalyptus oil, 1% pet., 25 controls negative), the sole ingredient of the cream eliciting a positive PT reaction (228)

Additional information:

ISO 4720:2009 nomenclature: *Eucalyptus globulus* Labill. Eucalyptus oils are produced from plants belonging to the genus *Eucalyptus* (*Myrtaceae*), which includes about 500 species in Australia, the country of origin, alone. At present, few of the oils, which are used to characterise species, are commercially important (34). Some species are rich in 1,8-cineole (80-85% content). Other species contain less cineole, but 10-22% alpha-pinene. *E. citriodora* predominantly contains citronellal (min. 75% by GC), with some citronellol and isopulegol (5-10% each) (34). *E. dives* contains (-)-piperitone and 15-25% alpha-phellandrene (34). According to (30) the maximum observed concentration in eucalyptus oil are (in %): 1,8-cineole (58; 70-80 after rectification); α -pinene (22); limonene (8); para-cymene (5); trans-pinocarveol (5); aromadendrene (10); globulol (2.5) [the latter 2 components only traces after rectification] (30).

For Crude or rectified oils of *Eucalyptus globulus* (*Eucalyptus globulus* Labill.) an ISO standard exists: ISO 770:2002.

It is a "top 100" substance and classified as R43 (IFRA, pers. comm.2010).

EUGENIA CARYOPHYLLUS LEAF / FLOWER OIL

CAS 8000-34-8; EC / (Oils, clove)

Clove oil

INCI: EUGENIA CARYOPHYLLUS OIL

Current regulation: /

Clinical data:

In the Larsen 2002 c study, 19.3% of patients with known contact allergy to fragrance ingredients reacted positively to "clove bud oil" (10 % pet.) (1). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "clove oil" were observed (3). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with clove oil (2% pet.) 13.4% positive reactions (9). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 1.6% positive reactions 2% pet. (22). The Rudzki 1976 study found 2 positive reactions in 200 patients to "clove" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=12 (13.3%) positive reactions to "clove" essential oil 2% pet. (27). The IVDK 2010 c study identified 1.5% positive reactions 6893 patients tested in the context of a special series (30).

A 32 year old Spanish physiotherapists developed vesicular hand dermatitis after using a "balsam from ash extract" cream. PTing revealed positive reactions to this cream, the FM I, eugenol, and 2 components of the cream: cinnamon oil (0.5% pet.) and clove oil (1% pet.) (225).

Additional information:

ISO 4720:2009 nomenclature: *Syzygium aromaticum* (L.) Merr. & L. M. Perry syn. *Eugenia caryophyllus* (Spreng.) Bullock & S. G. Harrison. Standards regarding the composition of clove oil are available: ISO 3141:1997, ISO 3142:1997, ISO 3143:1997. Clove oils are produced from the clove tree *Syzygium aromaticum* (L.) Merr. et L.M. Perry [*Eugenia caryophyllus* (Spreng.) Bullock ex S.G. Harrison]. The content of clove bud, clove leaf and clove stem oil has, with little variation, been determined by GC as 75-92% eugenol, 2-17% caryophyllene and 0.2-15% eugenyl acetate – the latter compound found in particularly high concentration in bud oil (34). According to another source, the following maximum content (%) has been observed regarding the constituents listed: eugenol (92,0);

caryophyllene (17); eugenyl acetate (15); isoeugenol (0.5) (30).

In a LLNA study by RIFM, the clove leaf oil as used was reported to contain 85.3% eugenol, 9.9% caryophyllene and 2.2% alpha humulene, according to analyses of the supplier. The EC3 value was calculated to be 7.1% (227).

EVERNIA FURFURACEA LICHEN EXTRACT

CAS 90028-67-4; EC 289-860-8
(*Evernia furfuracea*, ext. = INCI)

Tree moss extract

Current regulation: /

Clinical data:

The Larsen 1977 study in 20 "perfume-sensitive patients" yielded n=6 positive reactions with "treemoss abs. in benzyl benzoate, 5% petrolatum" (18). In the IVDK 2007 study, 2.7% (95% CI: 2.0 – 3.6%) of 1658 consecutive patients had a positive reaction to "tree moss absolute" (4). In the Groningen 2009 study, 2.5% (95% CI: 1.1 – 4.9%) had positive reactions to the allergen, tested at 2%, i.e., twice the commonly used concentration, and not in pet., but in diethylphthalate (6). The IVDK 2010 study, 6.02% (95% CI: 4.90 – 7.14%; percentages standardised for age and sex) of 1947 patients PTed reacted to the compound (7).

Additional information:

Syn.: *Pseudoevernia furfuracea* (L.) Zopf (53). The lichen grows on the bark of pine and fir trees. The extraction process with carbohydrate solvents yields a "concrete" (2-5% yield) which, in a next step eliminating waxy compounds, is extracted with warm alcohol and subsequent cooling, yielding an "absolute" (40-60% yield) (53).

EVERNIA PRUNASTRI

CAS 90028-68-5; EC 289-861-3
(*Evernia prunastri*, ext. = INCI)

Oak moss abs.

Current regulation: Annex III, part 1, n° 91

Clinical data:
In the "background information" section of the 1999 opinion, oak moss extract is classified as "most frequently reported allergen"; in consecutive PT patients, about 2.8% positive reactions had been reported (33). 'The German MAK commission has labelled oak moss extract as 'sensitising to the skin' (229).

Since the last SCCNFP-opinion of 1999, a "polymer based method" was developed to reduce the natural content of these two compounds from around 1 - several percent to < 75 ppm for atranol and < 25 ppm for chloratranol. However, PTing 14 subjects with previous positive PT reactions to the "oak moss" allergen preparation with the modified *Evernia prunastri* material still elicited positive reactions in 8/14 subjects; thus, the reduction in allergen content was deemed unsafe for the consumer (230). In a study of 885 consecutive eczema patients tested in Gentofte, Denmark, 3.2% had a positive or follicular patch test response to oak moss absolute. Two types of oak moss absolute were tested, one contaminated by resin acids and one without any detectable resin acids. There was no difference in reactivity between the two types of oak moss absolute

(231). The IVDK 2007 study yielded 2.2% (95% CI: 1.6 – 3.0%) positive reactions in 2063 consecutively tested patients (4). In the Groningen 2009 study, 1.9% (95% CI: 0.7 – 4.0%) had positive reactions to oak moss, tested at 2% pet., i.e., twice the commonly used concentration (6). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reaction (13) (test concentration 2% pet.). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded 5.0% positive reactions (22). The IVDK 2010 study, 1.81% (95% CI: 1.07 – 2.56%) of 1213 consecutively tested patients reacted to the compound, while 5.59% (95% CI: 4.90 – 6.27%) of 4482 of patients tested in a more aimed manner, partly as breakdown testing to the FM I, had a positive PT reaction (7). In a study from Alicante, Spain, 86 selected patients were tested with *E. prunastri* extract, yielding 2 positive reactions (48).

L. Kanerva et al. report on a 41 year old female hairdresser in whom oak moss abs. contained in a perming solution (concentration in the product unknown) was unequivocally identified as allergen causing (i) occupational hand dermatitis and (ii) scalp dermatitis after application to the own hair (232). Another case of occupational hand dermatitis in a grinding engineer was, at least partly, attributable to contact sensitisation to "oak moss resin" contained in a soluble oil (233).

Additional information:

Source: *Evernia prunastri* (Oak moss) (*Evernia prunastri* var. *prunastri* L. Ach). Oak moss is extracted as described above. Chloratranol and atranol are the degradation products of chloratranorin and atranorin, resp., which are recognised as the main sensitisers in *Evernia prunastri* extracts.

ILLICIIUM VERUM FRUIT OIL

CAS 84650-59-9, 8007-70-3; EC 283-518-1

"Anise Oil", Star anise oil

(Star anise, *Illicium verum*, ext. = INCI)

Current regulation: /

Clinical data:

In a study involving 100 consecutive patients, Rudzki and Grzywa found (i) a relatively high frequency of active sensitisation to star anise oil (n=5) tested with 0.5, 1 and 2% concentration (most likely in yellow petrolatum, as the other allergens in this series). Later patch testing with constituents of this essential oil (1%) in 3 patients yielded positive results to anethole in 3 cases, and to alpha-pinene and safrole in the 1 case tested to these substances. 34% of the consecutive patients reacted positively to star anise oil at 1%, which was considered as (marginally) non-irritating PT concentration (234).

Additional information:

ISO 4720:2009 nomenclature: *Illicium verum* Hook. f. *Illicium Verum* Fruit Oil is an essential oil distilled from the fruits of the Star Anise, *Illicium verum*, Illiciaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40297>, last accessed 2010-01-29). The main component is trans-anethole (86-93%), which can be purified from star anise oil. Main uses are alcoholic beverages, food flavouring and oral care products (34, 39). For Oil of star anise, Chinese type (*Illicium verum* Hook. f.) an ISO standard exists: ISO 11016:1999.

JASMINUM GRANDIFLORUM FLOWER EXTRACT	CAS 84776-64-7; EC 283-993-5 (Jasmine, <i>Jasminum grandiflorum</i> , ext. = INCI)
<i>Jasmine abs.</i>	
JASMINUM OFFICINALE FLOWER OIL	CAS 90045-94-6; EC 289-960-1 (Jasmine, <i>Jasminum officinale</i> , ext. = INCI)
JASMINUM OFFICINALE OIL	CAS 8022-96-6; EC / (Oils, jasmine) INCI: JASMINUM OFFICINALE OIL

Current regulation: /

Clinical data:

In the Frosch 2002 b study, a total of 1.2% of 1606 consecutive patients had a positive PT to "jasmine absolute", tested 5% in pet. (17). The deGroot 2000 study yielded 13 positive reactions to "jasmine, synthetic" in 1825 consecutively tested patients (12). In the early Larsen 1977 study, 18 of 20 "perfume sensitive patients" reacted to "Jasmin synthetic" 10% pet. (18), while 7 reacted to "Jasmin absolute" (10% pet.) – all of these also positive to the synthetic fragrance. The Sugiura 2000 study set in Nagoya, Japan, yielded 1% positive PT reactions in 1483 patients PTed for suspected cosmetic dermatitis, using 5% pet. as test concentration (14). The Larsen 2001 study in 178 patients with known contact allergy to fragrance ingredients found 16.9% positive reactions to jasmine absolute (10% pet.) (19). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had a positive reaction to Jasmine officinale oil (Jasmine absolute, Egyptian), tested at 2% (13). In the NACDG 2009 study, 1.1% of 4447 patients tested with "Jasmine absolute 2% pet." were found PT-positive (21). The Belsito 2006 study (20) yielded 0.4% positive reactions to "jasmine absolute". The Goossens 1997 study found 5 of 111 patients positive to "jasmine absolute" (10% pet.) – all sensitised to other fragrance allergens (23). In 63 patients positive to the FM I, 13 had positive PT reactions to "jasmine absolute", 2% pet., and 12 to "jasmine synthetic", 2% pet. in the Santucci 1987 study – the amount of concomitant reactivity was not examined (28). Nakayama et al. found 1974 (after (29)) 19 "strong positive" and 25 "weak positive" reactions to "jasmin oil" (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 1.5% positive reactions in 3668 consecutively tested patients and 1.2% positive reactions in 982 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were tested with jasmine absolute, yielding 3 positive reactions, and with "Jasmine synthetic", also resulting in 3 positive reactions (48).

Additional information:

Jasminum Grandiflorum Flower Extract is an extract obtained from the flowers of the Spanish Jasmine, *Jasminum grandiflorum* L., Oleaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39752>, last accessed 2009-11-12).

Jasminum Officinale Flower Oil is an essential oil obtained by molecular distillation of the flowers from the Jasmine, *Jasminum officinale* L., Oleaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39754>, last accessed 2009-11-25).

Jasminum Officinale Oil is the volatile oil obtained from the flowers of the Jasmine, *Jasminum officinale* L., Oleaceae

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=34776>, last accessed 2010-01-05); this latter extract is used by Almirall/Hermal/Trolab for the preparation of a PT allergen.

Jasmine absolute is obtained by solvent extraction, via concrete, from the flowers of *J. grandiflorum* (L.) Aiton from China and India. The main volatile compound is benzyl acetate, however, minor compounds such as indole [120-72-9], cis-jasmone [488-10-8] and methyl jasmonate [1211-29-6] contribute to the typical jasmine fragrance (34). Reported compounds include the following (maximum observed concentration given in parentheses): benzyl acetate (28); benzyl benzoate (24.0); phytol acetate (9); isophytol (8.5); phytol (7.4); linalool (7.0); eugenol (4.0); squalene (4); indole (3.5); benzyl alcohol (2.5); cis-jasmone (2.5); methyl linolenate (2.0); methyl palmitate (1.4); p-cresol (1.0); cis-3-hexenyl benzoate (1.0); benzyl salicylate (0.4); jasmin lactone (0.9); methyl jasmonate (0.7); isoeugenol (0.4) ((30), also according to (17))

JUNIPERUS VIRGINIANA OIL

CAS 8000-27-9; EC / (Oils, cedarwood) [this also refers to *Cedrus atlantica* ...] / 85085-41-2; EC 285-370-3 (*Juniper*, *Juniperus virginiana*, ext. = INCI)

JUNIPERUS VIRGINIANA WOOD OIL

CAS 85085-41-2; EC 285-370-3

Cedar Wood Oil (Virginian)

Current regulation: /

Clinical data:

In the Frosch 2002 b study, a total of 0.6% of 1606 consecutive patients had a positive PT to "cedarwood oil (Moroccan and Chinese 1:1)", tested 10% in pet. (17). After application of Penaten-baby™ oil as immersion oil for dermatoscopy a patient developed multiple patches of eczema at the application sites. Investigation revealed that the oil was kept in a bottle previously used for *Juniperus virginiana* oil, to which contact sensitisation was verified by patch testing (235).

Additional information:

ISO 4720:2009 nomenclature: *Juniperus virginiana* L.. *Juniperus Virginiana* Oil is the volatile oil obtained from the fruits and leaves of the Red Cedar, *Juniperus virginiana* L., Cupressaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=78070>, last accessed 2010-01-05)

Juniperus Virginiana Wood Oil is an essential oil obtained from the wood and twigs of the Red Cedar, *Juniperus virginiana* L., Cupressaceae. It contains chiefly (alpha and beta) cedrene and cedral (cedar camphor), cuparene, thujopsene, widdrol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39767>, last accessed 2009-11-12)(235). According to Surburg/Panten by GC: alpha-cedrene 22-35%, thujopsene 10-25%, cedrol 16-25% (34).

See also *Cedrus atlantica*. According to (30) the maximum observed concentration in cedar wood oil are (in %): α-cedrene (32); thujopsene (25); cedrol (25); β-cedrene (6); widdrol (5) and cuparene (traces) (30).

For Oil of cedarwood, Virginian (*Juniperus virginiana* L.) an ISO standard is available: ISO 4724:2004. For Oil of cedarwood, Texas (*Juniperus mexicana* Schiede) an ISO standard exists: ISO 4725:2004.

LAURUS NOBILIS OIL

CAS 8002-41-3; EC / (Oils, laurel)
 INCI: LAURUS NOBILIS OIL /
 8007-48-5; EC / (Oils, sweet
 bay)/ 84603-73-6; EC 283-272-5
 (Laurus nobilis, ext.) INCI:
 LAURUS NOBILIS EXTRACT

Laurel oil

Current regulation: Annex II, n° 359 (seed oil)

Clinical data:

In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=4 (0.6%) positive reactions to "laurel oil" 2% pet. (22).

After sensitisation by a one-time occlusive application a 36 year old Turkish patient developed widespread allergic contact dermatitis 3 days after massage with olive oil containing Laurus nobilis oil; sensitisation was proven by a strong positive reaction to the commercial test preparation and the massage oil previously used (236). Topical application of laurel oil for knee arthropathy led to an erythema exudativum multiforme-like rash on the legs of a 63 year old patient; interestingly, laurel oil yielded a "target like" strongly positive PT reaction in this case (237). In an earlier Turkish case with a similar history, the EEM-like appearance was lacking; however, a very intense, edematous reaction was noted (238). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "laurel oil" were observed (3). The IVDK 2010 c study identified 1.0% positive reactions in 6297 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Laurus nobilis* L. Laurel leaf oil is obtained by steam distillation of leaves from Laurus nobilis L. (Lauraceae), an evergreen cultivated primarily in the Mediterranean countries. The main components are 1,8-cineole (30-70%), linalool (about 10%) and eugenol (34). According to (30) the maximum observed concentration in laurel oil are (in %): 1,8-cineole (70); β -caryophyllene (11); linalool (11); limonene (5.0); eugenol (2.0); geraniol (0.3) (30).

LAVANDULA HYBRIDA HERB OIL

CAS 91722-69-9; EC 294-470-6
 (Lavender, Lavandula hybrida,
 ext. = INCI)

Lavandin Oil

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "lavandin" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to "lavandin" essential oil 2% pet. (27). In the Frosch 1995 dose-finding pilot study, no positive reaction to 1% and 5% lavandin oil in pet., tested in 205 consecutive patients in Dortmund and Göttingen, and just 1 irritant reaction to

the higher concentration, were observed (15).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula angustifolia* Mill. x *Lavandula latifolia* Medik. *Lavandula* *hybrida*, *Labiatae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=39789>, last accessed 2010-01-29. Nomenclature according to Surburg/Panten: *Lavandula* x *intermedia* Lois, which is a hybrid of lavender and spike (see below) (34). The oils from the most important variants, abrial and grosso, contain linalool (24-38%), linalyl acetate (20-38%) as well as 1,8-cineole (4-11%), and camphor (6-11%) (34). A third variant is called super because of its high concentration of linalyl acetate (35-47%), more closely resembling lavender oil (34). For Oil of lavandin Grosso (*Lavandula angustifolia* Mill. x *Lavandula latifolia* Medik.), French type an ISO standard exists: ISO 8902:2009, for Oil of lavandin Abrial (*Lavandula angustifolia* Miller x *Lavandula latifolia* Medikus), French type a different ISO standard: ISO 3054:2001.

It is a "top 100" substance (IFRA, pers. comm.2010)

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

LAVANDULA OFFICINALIS FLOWER OIL

CAS 84776-65-8, 8000-28-0; EC 283-994-0 (*Lavender*, *Lavandula angustifolia angustifolia*, ext. = *INCI*)

Lavender oil

Current regulation: /

Clinical data:

In a large series from Nagoya, Japan, 1483 patients were tested with lavender oil 20% in pet., with overall 3.7% positive reactions from 1990 to 1998. However, within this period, a sharp increase was noted in 1997 and 1998, which as attributed to changed exposure by M. Sugiura et al. (14). On the individual level, relevance of positive reactions remained unclear in about half of the cases. The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "lavender absolute" (2% pet.) 6.6% positive reactions (9). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to "Lavandula augustifolia oil" (Lavender absolute) 2% (13). The Goossens 1997 study found 4 of 111 patients positive to "lavender oil 20% pet." – all of them sensitised to other fragrance allergens (23). The Rudzki 1976 study found no positive reaction in 200 patients to "lavender" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "lavender" essential oil 2% pet. (27). Nakayama et al. found 1974 (after (29)) 6 "strong positive" reactions to "Lavender oil" (unknown test concentration) in 183 patients. In a study from Alicante, Spain, 86 selected patients were tested with "lavender absolute", yielding 2 positive reactions (48).

R. Goiriz et al. report on a case of photo contact allergy (10 controls negative) in a 45 year old woman developing after application of a ketoprofen-containing topical gel ("Fastum") (239). A physiotherapist developed acute, recurrent dermatitis after use of "Diffiam® gel", scented with lavender oil. Both the gel and lavender oil (2% pet.) tested positive; avoidance resulted in clearing (240). In a study on 218 patients with known

contact allergy to fragrance ingredients, Larsen (2002 c) found positive reactions to 10% lavender oil (pet.) in 2.8% of these (1). A case of vulvovaginitis with spread and affecting the dominant hand applying various tea tree and lavender oil creams was reported by S. Varma; the PT with 10% lavender oil abs. in pet. (50 controls negative) was positive (241). In two cases, facial "pillow dermatitis" due to lavender oil, applied to the pillows, developed, confirmed by positive PT to lavender abs. (2% pet.) (242).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula angustifolia* Mill. *Lavandula officinalis* Flower Oil is an essential oil obtained from the fresh flowering tops of the Lavender, *Lavandula officinalis* (syn: *L. vera*), *Labiatae*. It contains 30-40% esters calculated as linalyl acetate, linalool, pinene, limonene, geraniol, some eucalyptol (cineol) (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40370>, last accessed 2009-11-09). According to Surburg/Panten, lavender oil is obtained by steam distillation of freshly cut flowering tops of *Lavandula angustifolia* Mill. (Lamiaceae). Main constituents according to GC are linalyl acetate (25-45%), cis-ocimene (4-10%), trans-ocimene (1.5-6%), 1,8-cineole ($\leq 1\%$) camphor ($\leq 0.5\%$), linalool (25-38%), 1-terpinen-4-ol (2-6%) and lavandulyl acetate [25905-14-0] ($\geq 2\%$) (34).

In addition to distillation, both *Lavandula officinalis* and Lavandin are also solvent extracted, yielding concretes and, after ethanol extraction, absolutes, which are said to have a longer-lasting odour (34).

For Oil of lavender (*Lavandula angustifolia* Mill.) an ISO standard exists: ISO 3515:2002.

LAVANDULA SPICA HERB OIL

CAS 97722-12-8; EC 307-762-6
(Lavender, *Lavandula spica*, ext.
= INCI

"Spike Oil"

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "spike" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=8 (9.3%) positive reactions to "spike" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Lavandula latifolia* Medik. *Lavandula Spica* Herb Oil is an essential oil distilled from the flowering herbs of the Spikenard, *Lavandula spica* (syn: *Lavandula latifolia*), *Labiatae*. It contains eucalyptol (35%), camphor, linalool, borneol, terpineol, D-camphene and sesquiterpenes (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40372>, last accessed 2010-01-29). According to Surburg/Panten, Spanish spike lavender oil is steam distilled from the flowering tops of *Lavandula latifolia* Medik.. The main components are linalool (34-50%), 1,8-cineole (16-39%) and camphor (8-16%) (34). For Oil of spike lavender (*Lavandula latifolia* (L.f.) Medikus), Spanish type an ISO standard exists: ISO 4719:1999

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

LITSEA CUBEBA FRUIT EXTRACT

CAS 90063-59-5, 68855-99-2; EC
290-018-7 (*Litsea cubeba*, ext.)
INCI: LITSEA CUBEBA OIL

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 3 positive reaction in 200 patients to "Litsea cubeba" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=7 (8.1%) positive reactions to this essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Litsea cubeba* (Lour) Pers. Litsea Cubeba Fruit Extract is an extract obtained from the fruits of the plant, *Litsea cubeba*, Lauraceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40036>, last accessed 2009-11-24. The content by GC is: neral (25-33%), geranial (38-45%) – i.e. about ¾ citral, for which the extract had previously served as a raw material (34); direct use for perfuming is limited to household products (39). For Oil of Litsea cubeba (*Litsea cubeba* Pers.) an ISO standard exists: ISO 3214:2000.

In a LLNA study by RIFM, the "Litsea cubeba oil" as used was reported to contain 85.7% citral, 2.9% limonene, 1.7% linalool, 1.4% citronellal and < 1% caryophyllene and methyl heptanone, according to analyses of the supplier. The EC3 value was calculated as 8.4 % (227).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

MENTHA ARVENSIS LEAF OIL

CAS 68917-18-0 ; EC /

Cornmint oil

INCI: MENTHA ARVENSIS OIL

Current regulation: /

Clinical data: /

Additional information:

Mentha Arvensis Leaf Oil is the oil derived from the leaves of the Horse Mint, *Mentha arvensis* L., Labiatae (http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=57860)

It is a "top 200" substance and classified as R43 (IFRA, pers. comm.2010)

MENTHA PIPERITA OIL

CAS 8006-90-4; EC / (Oils,
peppermint) INCI: MENTHA

*PIPERITA OIL / 84082-70-2; EC
282-015-4 (Peppermint, ext.) INCI
names: MENTHA PIPERITA ...*

Peppermint oil

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.6% of 1606 consecutive patients reacted positively to "peppermint oil (American)", tested 2% in pet. (17). In a series of 40 of 744 consecutive patients PTed with an extended fragrance series (Sheffield 1999), 2 positive reactions to "peppermint oil" were observed (3). In the Wöhrle 2001 study, PTing 747 patients with suspected contact allergy to fragrance ingredients yielded n=1 (0.1%) positive reactions to peppermint oil 2% pet. (22). Among 512 patients referred from a dental department for diagnostic work-up of various intraoral symptoms and complaints within 4 years, 6 patients had positive (+ to +++) PT reactions to "peppermint oil" 1% pet. at D4, mostly combined with positive reactions to menthol (see above) and reporting dramatic improvement after cessation of use of peppermint-containing oral products (154). The Rudzki 1976 study found 1 positive reaction in 200 patients to "Peppermint" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=6 (6.9%) positive reactions to "peppermint" essential oil 2% pet. (27). In 63 patients positive to the FM I, 3 had positive PT reactions to peppermint oil, 2% pet., in the Santucci 1987 study (28). The IVDK 2010 c study identified 0.6% positive reactions in 6546 patients tested in the context of a special series (30).

An unusual case of "baboon-like" allergic contact dermatitis of the vulva after drinking excessive amounts of a herbal tea containing, among other ingredients, peppermint. While the PT reaction to peppermint oil was only weak to doubtful, dramatic improvement after cessation and prompt relapse after repeat ingestion proved the diagnosis (243). Recurrent foot and lower leg dermatitis after the application of a "foot spray" (containing peppermint oil) was diagnosed as allergic contact dermatitis due to this ingredient in a 59 year old golf player (244). In another case, ACD after application of a transdermal system for the treatment of lumbar pain was attributed to CA to peppermint oil (2% pet.) and its main ingredient menthol (1% pet.) (155). In a patient with toothpaste-induced cheilitis, not only *M. piperita*, but also *M. arvensis*, but not *M. spicata* or *cardica* extracts (all tested 1% pet.), as well as natural and synthetic menthol caused positive PT reactions (245).

Additional information:

ISO 4720:2009 nomenclature: *Mentha x piperita* L. A standard by ISO exists for Oil of peppermint (*Mentha x piperita* L.): ISO 856:2006. A review by the Cosmetic Ingredient Review Expert Panel, Washington, DC on the "Final report on the safety assessment of *Mentha Piperita* (Peppermint) Oil, *Mentha Piperita* (Peppermint) Leaf Extract, *Mentha Piperita* (Peppermint) Leaf, and *Mentha Piperita* (Peppermint) Leaf Water" is available (163), stating that "Peppermint Oil is used at a concentration of < or = 3% in rinse-off formulations and < or = 0.2% in leave-on formulations. Peppermint Oil is composed primarily of menthol and menthone. Other possible constituents include pulegone, menthofuran, and limone. According to Surburg/Panten: (-)-menthol (34-46%), (-)-menthone (15-27%), (-)-menthyl acetate (2.5-7%) and menthofuran [17957-94-7] (0.5-6%) (34). According to (30) the maximum observed concentration in peppermint oil are (in %): (-)-menthol (49); (-)-menthone (28); (-)-menthyl acetate (8); mentofuran (8); isomenthone (8); neo menthol (6); pulegone (3.5); limonene (3.0); linalool (0.4) (30). Most of the safety test data concern Peppermint Oil. The oil is considered to present the "worst case scenario" because of its many constituents, so data on the oil were considered relevant to the entire group of ingredients. ... Repeated

intradermal dosing with Peppermint Oil produced moderate and severe reactions in rabbits" concluding that "with the limitation that the concentration of pulegone in these ingredients should not exceed 1%, it was concluded that Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Extract, Mentha Piperita (Peppermint) Leaves, Mentha Piperita (Peppermint) Water are safe as used in cosmetic formulations".

MENTHA SPICATA HERB OIL

CAS 84696-51-5, 8008-79-5; EC 283-656-2 (Spearment, ext.)

Spearment oil

INCI: MENTHA VIRIDIS EXTRACT

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.8% of 1606 consecutive patients reacted positively to "spearment oil (American)", tested 2% in pet. (17). The CAS # quoted (8008-79-5) refers, according to CosIng, to MENTHA VIRIDIS LEAF OIL, the volatile oil obtained from the dried tops and leaves of the Garden Mint, *Mentha viridis* L., Labiatae. The Larsen 2001 study diagnosed 5.0% positive reactions in 178 patients with known contact allergy to fragrance ingredients, using this oil at 5% pet. test concentration (19). In the An 2005 study, 6 of 422 consecutive patients, i.e., 1.4%, had positive reactions to "Mentha viridis oil" 5% (13). PT results with toothpaste ingredients were positive in 7 patients, of whom 4 had strong positive reactions to spearment (246).

Additional information:

ISO 4720:2009 nomenclature: *Mentha spicata* L. *Mentha Spicata* Oil is an essential oil obtained from the herbs of the Spearment, *Mentha spicata* L., Labiatae (syn: *Mentha viridis* L., Labiatae). It contains carvone (more than 50%), limonene, pinene (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40394>, last accessed 2009-11-11). According to Surburg/Panten, the content is limonene (9-16.5%), (-)-carvone (60-70%), menthone (0-0.2%) and viridiflorol (0-0.5%) (34). Exposure by toothpastes, and subsequent contact allergic reaction of the lips or the oral mucosa, have been reported (e.g., (247, 248)). L-Carvone is a component of the oil from *Mentha spicata* (spearment) (53) and had been tested with positive results in "toothpaste cases", even at a concentration as low as 0.067% (68).

For Oil of spearment -- Part 1: Native type (*Mentha spicata* L.) an ISO standard exists: ISO 3033-1:2005, for Oil of spearment -- Part 2: Chinese type (80 % and 60 %) (*Mentha viridis* L. var. *crispa* Benth.), redistilled oil: ISO 3033-2:2005, for Oil of spearment -- Part 3: Indian type (*Mentha spicata* L.), redistilled oil: ISO 3033-3:2005 and for Oil of spearment -- Part 4: Scotch variety (*Mentha x gracilis* Sole): ISO 3033-4:2005.

MYROXYLON PEREIRAE RESIN

CAS 8007-00-9; EC 232-352-8 (Balsams, Peru)

Balsam of Peru

INCI: MYROXYLON PEREIRAE / Balsams, Peru

Current regulation: Annex III, part1, n° 154

Clinical data:

This natural mixture has been employed as screening agent in Baseline series worldwide for many decades. Hence, a wealth of data is available; table 3.2 – 1 summarises results of the past 10 years.

Additional information:

ISO 4720:2009 nomenclature: *Myroxylon pereirae* (Royle) Klotzsch, syn. *Myroxylon balsamum* var. *pereirae* (Royle) Harms. *Myroxylon pereirae* resin (MPR, Balsamum peruvianum) is harvested from the balsam of Peru tree, *Myroxylon balsamicum* (L.) HARMS var. *pereirae* (ROYLE) HARMS, synonymous *Myroxylon pereirae* (ROYLE) KLOTZSCH (249) after thermal stress, almost exclusively in El Salvador. Main constituents of the pleasantly, vanilla-like smelling dark brown liquid are benzyl esters of cinnamic and benzoic acid (35 – 75%), up to 30% cinnamic acid, up to about 10% benzoic acid, approximately 5% alpha- and beta-nerolidol, benzyl alcohol and mostly less than 1% cinnamyl alcohol, benzyl ferulate and -isoferulate, cinnamic acid amyl ester, coniferyl alcohol, coniferyl benzoate, eugenol, isoeugenol, farnesol, vanillin, and several trace constituents (250-253). The composition of MPR varies with the origin and other factors; moreover, MPR is sometimes blended with other natural mixtures such as turpentine, styrax or colophonium (249).

MPR can be used to improve taste or smell in gargling solutions, cosmetic products such as soaps, shampoo or lipsticks, as well as sweets, tobacco and beverages (249, 254). According to EU legislation and IFRA guidelines MPR should not be used in products intended for skin contact; however, extracts and distillates of MPR may be used in a concentration of < 0,4% (IFRA-Guidelines, www.ifraorg.org (255)). E. Temesvári et al. report on the interesting case of severe ACD with subsequent hypopigmentation after a "temporary henna tattoo", which was, unexpectedly, not due to p-phenylene diamine, but to the oil used to disperse the pigment, which presumably contained allergens also included in the FM I and MPR, both of which were extreme positive on a later PT (256).

In addition to delayed type hypersensitivity reactions, MPR (and some of his constituents such as benzoic acid (257)) are capable of eliciting (non-immunological) urticarial immediate reactions (258-260). In one case, the immediate reaction to MPR (and to FM I) at the test site spread systemically in terms of a generalised urticaria, while no delayed type reactions were observed to the PT (261). Generally, there is apparently no association of immediate reactions to MPR (and cinnamal or cinnamyl alcohol) and contact sensitisation to these compounds (262). In animal experiments the sensitising potency of MPR was clearly established (250), with coniferyl benzoate identified as single compound with the most marked potency (252). However, due to the limited chemical stability of this compound it is unclear whether other, more stable compounds are, in fact, more important allergens, such as cinnamic acid and (iso-) ferulic acid esters or oxidised constituents of the resin fraction (263).

Table 3.2.2 – 1: Results with contact allergy to fragrance ingredients screening agents reported since 1999 in patients patch tested for suspected allergic contact dermatitis: **Myroxylon pereirae resin** (Balsam of Peru) 1). If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the reviewers.

Country	Population	Years	No. tested	Crude % positive (95% CI) §
Tel Aviv, Israel (264) #	Consecutive patients	1999-2000	943	6.6 % (5.1 – 8.4) §
South Korea (13)	Consecutive patients	04/2002 – 06/2003	422	7.3% (5.1 – 10.3%) §

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Tel Aviv, Israel (265)	Consecutive patients	1998-2004	2156	3.6 (2.9 – 4.5) § %
Manipal, India (266)	Dermatitis patients	1989-1998	1780	n=17
Tehran, Iran (267)	Consecutive patients	2002-2004	250	2.4 (0.9 – 5.2) § %
Sevilla, Spain (268)	Consecutive patients	2002-2004	863	5.8 (4.3 – 7.6) § %
Ankara, Turkey (269)	Consecutive patients	1992-2004	1038	2.1 (1.3 – 3.2) § %
Vienna, Austria (22)	Consecutive patients of one clinic	1997-2000	2660	5.4% (4.6 – 6.3%) §
Czech Republic (270)	Consecutive patients	1997-2001	12058	7.3% (6.8 – 7.8) §
Copenhagen, Denmark (271)	Consecutive patients	1985-2007	16173	3.9 (3.6 – 4.2) § %
Sweden (272)	Consecutive patients	2000	3790	6.5%
9 European countries (273) §	Consecutive patients	2002-2003	9672	6.1 %
Germany, 3 Swiss + 1 Austrian Dept. (7)	Consecutive patients	2005-2008	36919	8.0% (7.7 – 8.3%)
10 depts. From 7 EU countries (274) *	Consecutive patients	1996-2000	26210	6.0 %
USA (Canada) (20)	Probably consecutive patients	2003	1603	6.6%
NACDG 2009 (21)	Consecutive patients	2005-2006	4449	11.9%

§ Calculated by reviewers, where possible (if actual numbers were given)

Probably included in (265)

\$ > 5-fold difference between departments

* About 4-fold difference between departments

NARCISSUS SPP. EXTRACT / OIL

CAS: diverse

Narcissus abs.

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 1.3% positive reactions to "narcissus absolute" (2% pet.) were observed in 1606 consecutive (17). The extract used by the PT allergen provider Almirall/Hermal/Trolab has the CAS number 90064-25-8. The IVDK 2010 c study identified 0.5% positive reactions in 2445 consecutively tested patients and 0.6% positive reactions in 809 patients tested in the context of a special series (30).

Additional information:

Commonly used: *Narcissus poeticus* L. According to (30) the maximum observed concentration in Narcissus abs. are (in %): α -terpineol (23.7); trans-Isoeugenol methyl ether (20); benzyl benzoate (20); coumarin (5.7); benzyl alcohol (4.0); Δ^3 -carene (3.4); cinnamyl alcohol (2.5); phenylethyl alcohol (2.2); ethyl palmitate (2.2); phenylpropyl acetate (1.7); 1,8-cineole (1.5); caryophyllene (1.0); benzyl acetate (0.7); isoeugenol (0.5); farnesol (0.3) (also according to (17)) (30).

OCIMUM BASILICUM HERB OIL

CAS 84775-71-3; EC 283-900-8 (*Ocimum basilicum*, ext. = INCI)

Basil Oil (sweet)

Current regulation: /

Clinical data:

/

Additional information:

ISO 4720:2009 nomenclature: *Ocimum basilicum* L. For Oil of basil, methyl chavicol type (*Ocimum basilicum* L.) an ISO standard exists: ISO 11043:1998. *Ocimum Basilicum* Herb Oil is an essential oil obtained from the herbs of the Sweet Basil, *Ocimum basilicum* L., *Labiatae*. (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40474>, last accessed 2009-11-24). The chemical composition varies greatly with the origin (34):

- Basil oil of the methylchavicol type (Réunion type) is extracted from flowering tops or whole plants from Réunion, Comores, Madagascar, but also other countries such as Egypt. Mainly used for seasoning food. Content by GC: methylchavicol 75-87%, linalool 0.5-3%
- Basil oil, linalool type is produced mainly in the Mediterranean area. Content by GC: Linalool 45-62%, methylchavicol trace to 30%, eugenol 2-15%
- Indian Basil oil is produced exclusively in India. Content by GC: methylchavicol trace to 70%, linalool 25%.

In a LLNA study by RIFM, the basil oil as used was reported to contain 51% linalool, 10.4% eugenol, 7.7% cineol, 3.7% bergamotene, 2.7% germacrene D, 2.7% cadinol and 1.3% cadinene, according to analyses of the supplier. The EC3 value was calculated to be < 2.5% (227).

PELARGONIUM GRAVEOLENS FLOWER OIL

CAS 90082-51-2; EC 290-140-0 (*Pelargonium graveolens*, ext. = INCI) / 8000-46-2; EC / (Oils, geranium) INCI: GERANIUM

Geranium Oil Bourbon

Current regulation: /

Clinical data:

The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "geranium oil Bourbon" (2% pet.) 7.4% positive reactions (9). In the Larsen 2001 study, 8.4% positive reactions were observed in 178 patients with known contact allergy to fragrance ingredients ("geranium oil Bourbon", 10% pet.) (19). The Goossens 1997 study found 3 of 111 patients positive to "geranium oil 20% pet." – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found 3 positive reactions in 200 patients to "geranium" essential oil 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to "geranium" essential oil 2% pet. (27). Nakayama et al. found 1974 (after (29)) 3 "strong positive" reactions to "Geranium oil" (unknown test concentration) in 183 patients, Trattner/David 1 / 641 consecutive patients positive to "Geranium oil" (31). In a study from Alicante, Spain, 86 selected patients were patch tested with an extended fragrance series; n=8 reacted positively to geranium oil bourbon (48).

Additional information:

ISO 4720:2009 nomenclature: *Pelargonium x ssp.* For Oil of geranium (*Pelargonium X ssp.*) an ISO standard exists: ISO 4731:2006 *Pelargonium Graveolens Flower Oil* is the volatile oil obtained from the flowers of the Bourbon Geranium, *Pelargonium graveolens* L. Hér. Ex Aiton, *P. roseum* Willdenow (and other nondefined hybrids that have developed in different regions of the world) Geraniaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=57527>, last accessed 2009-11-16)(34). The Bourbon type (Réunion, Madagascar) is more valuable than the North African and Chinese products, and differs in characteristic components: (-)-6,9-guaiadiene [36577-33-0] 5-9% in the Bourbon type, and 10-epi-gamma-eudesmol [15051-81-7] 3-6% in the African type, in addition to the main components (-)-citronellol, isomenthone, formates and tiglates. Chinese oil is similar to Bourbon oil, however, it contains more citronellol (32-43%) and lower amounts of linalool (2-4.5%) and geraniol (5-12%) (34).

In a LLNA study by RIFM, the geranium oil as used was reported to contain 41.1% citronellol, 9.8% 2,6-guiadine, 6.2% isomethone, 4.9% geraniol, 2.2% cis-rose oxide, 2.1% linalool, 1.5% geranyl formate, 1.3% phenyl ethyl tiglate, 1.0% trans-rose oxide, and geranyl tiglate and alpha-pinene at < 1%, according to analyses of the supplier. The EC3 value was calculated to be > 50% (227).

PELARGONIUM ROSEUM LEAF OIL

CAS 90082-55-6; EC 290-144-2
(*Pelargonium roseum*, ext. =
INCI)

Geranium Oil; Rose Geranium Oil

Current regulation: /

Clinical data:

In the Sugiura 2000 study, among 1483 patients with suspected cosmetic dermatitis, 2.1% positive PT reactions to "geranium oil" (tested 20% in pet.) were observed (14).

Additional information:

Pelargonium Roseum Leaf Oil is an essential oil obtained from the leaves of the plant, Pelargonium roseum, Geraniaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40565>, last accessed 2009-11-16).

PIMENTA RACEMOSA LEAF/FRUIT OIL

CAS 85085-61-6; EC 285-385-5

Bay oil (34)

Current regulation: /

Clinical data:
/

Additional information:

ISO 4720:2009 nomenclature: *Pimenta racemosa* (Mill.) J.W. Moore. For Oil of bay [*Pimenta racemosa* (Mill.) J.W. Moore] an ISO standard exists: ISO 3045:2004 Pimenta Racemosa Leaf/Fruit Oil is an essential oil obtained from the fruits of the plant, Pimenta racemosa, Myrtaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41014>, last accessed 2010-02-10).

Steam distillation of the leaves of Pimenta racemosa (Mill.) J.W. Moore (Myrtaceae) yields bay oil, which consists of myrcene (20-30%), eugenol (42-56%) and chavicol (8-13%) (34).

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

Pinus mugo leaf and twig oil and extract

CAS 90082-72-7, 8000-26-8; EC 290-163-6

Dwarf pine needle oil
(German: Latschenkiefernöl)

Current regulation: Annex III, part 1, 109

Clinical data:

In the Frosch 2002 b study, 0.7% positive reactions to dwarf pine needle oil (2% pet.) were observed in 1606 consecutive (17). The Rudzki 1976 study found 4 positive reactions in 200 patients to "Pine needle" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "pine needle" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Pinus mugo* Turra syn. *Pinus montana* Mill.) Pinus Mugo Twig Oil is an essential obtained from the twigs of the Pine, Pinus mugo, Pinaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.h.details&id=41476&back=1>, last accessed 2010-03-09). Pinus Mugo Twig Leaf Extract is an extract obtained from the twigs leaves of the Pine, Pinus mugo, Pinaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.h.details&id=41476&back=1>, last accessed 2010-03-09).

[h.details&id=41473&back=1](#), last accessed 2010-03-09).

Dwarf pine needle oil is obtained from *Pinus mugo* Turra subsp. *mugo* and subsp. *pumilio* (Haenke) Franco (34). For Oil of dwarf pine (*Pinus mugo* Turra) an ISO standard exists: ISO 21093:2003. American pine oils contain almost no 3-carene or camphene (34).

PINUS PUMILA TWIG LEAF EXTRACT / OIL

CAS 97676-05-6; EC 307-681-6
(Pine, *Pinus pumila*, ext. = INCI)

Dwarf pine needle oil

Current regulation: Annex III, part 1, 114

Clinical data: /

Additional information:

Pinus Pumila Twig Leaf Extract obtained from the twigs leaves of the Pine, *Pinus pumila*, Pinaceae

(<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41483&back=1>, last accessed 2009-11-12), *Pinus Pumila* Twig Leaf Oil is the essential oil obtained from the twigs leaves of the Pine, *Pinus pumila*, Pinaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41484&back=1>, last accessed 2009-11-12). Main constituents are alpha-pinene (60-70%) and beta-pinene (20-25%). (34) Occurrence from Siberia to Japan, classified as Endangered Species

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

POGOSTEMON CABLIN OIL

CAS 8014-09-3; EC / (Oils, patchouli) / 84238-39-1; EC 282-493-4 (Patchouli, ext.)

Patchouli oil

INCI: POGOSTEMON CABLIN / Patchouli, ext.

Current regulation: /

Clinical data:

In the Frosch 2002 b study, 0.8% positive reactions to patchouli oil (10% pet.) in 1606 consecutive were observed (17). Nakayama et al. found 1974 (after (29)) 3 "strong positive" and 8 "weak positive" reactions to "Patchouli oil" (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 0.6% positive reactions in 2446 consecutively tested patients and 1.4% positive reactions in 828 patients tested in the context of a special series (30).

Additional information:

ISO 4720:2009 nomenclature: *Pogostemon cablin* (Blanco) Benth. syn. *Mentha cablin* Blanco. An ISO standard is available for Oil of patchouli (*Pogostemon cablin* (Blanco) Benth.): ISO 3757:2002. *Pogostemon Cablin* Leaf Oil is an essential oil obtained from the fermented leaves of the Patchouli, *Pogostemon cablin* (syn: *Pogostemon patchouli*),

Labiatae (Lamiaceae (34)). It contains patchouli alcohol, beta-patchoulene, azulene, eugenol, sesquiterpenes (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40927>, last accessed 2009-11-12). Although the sesquiterpene alcohol (-)-patchoulol [5986-55-0] is the main component of patchouli oil (27-35%), the compound largely contributing to the characteristic odour is norpatchoulol [41429-52-1] (0.35-1%). Other constituents include (+)-alpha-bulnesene [6391-11-0] (13-21%), (-)-alpha-guajene [3691-12-1] (11-16%), (-)-β-patchoulene [514-51-2] (1.8-3.5%) and (-)-seychellene [20085-93-2] (1-3%) (34). According to (30) the maximum observed concentration in patchouli oil are (in %): (-)-patchoulol (35); (+)-alpha-lulnesene (21); (-)-alpha-guajene (16); β-pinene (6); (-)-β-patchoulene (3.5); (-)-seychellene (3); pogostol (2.5); α-pinene (2.5); norpatchoulol (1) (30).

It is a "top 100" substance (IFRA, pers. comm.2010).

ROSE FLOWER OIL (ROSA SPP.)

CAS 8007-01-0; EC / (Oils, rose)

ROSA ALBA FLOWER EXTRACT

CAS 93334-48-6; EC 297-122-1
(Rose, *Rosa alba*, ext. = INCI)

ROSA CANINA FLOWER OIL

CAS 84696-47-9; EC 283-652-0
(Rose, *Rosa canina*, ext.) INCI:
ROSA CANINA

ROSA CENTIFOLIA FLOWER OIL

CAS 84604-12-6, EC 283-289-8
(Rose, *Rosa centifolia*, ext.) INCI:
ROSA CENTIFOLIA / Rose, *Rosa centifolia*, ext.

ROSA DAMASCENA FLOWER OIL

CAS 90106-38-0; EC 290-260-3
(Rose, *Rosa Damascena*, ext. = INCI)

ROSA GALLICA FLOWER OIL

CAS 84604-13-7; EC 283-290-3
(Rose, *Rosa Gallica*, ext.) INCI:
ROSA GALLICA

ROSA MOSCHATA OIL

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ROSA RUGOSA FLOWER OIL

CAS 92347-25-6; EC 296-213-3
(Rose, *Rosa rugosa*, ext.)

Current regulation: /

Clinical data:

In the Sugiura 2000 study, 1483 patients with suspected cosmetic dermatitis were PTed with "rose oil Bulgaria" (2% pet.), yielding 0.4% positive reactions (14); Trattner/David found 2 / 641 consecutive patients positive to "Rose oil (Bulgarian)" (31). The Bulgarian rose oil usually corresponds to *Rosa Damascena* Flower Oil (http://en.wikipedia.org/wiki/Rose_oil, last accessed 2009-11-16). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "rose Bulgarian oil" (2% pet.) 4.5% positive reactions (9). One case of contact allergy to "Bulgarian rose oil (2 % pet.)" – and geraniol – in a 48-year-old female with ACD after application of "Eau de Rochas" E.d.C. was diagnosed, among 326 patients with suspected contact allergy to fragrance ingredients had tested negative (275). However, other rose oils are also used (and capable of eliciting ACD) as illustrated by the case of a 27 year old woman who developed ACD after using "Rose Absolute Eau ® eau de

parfum", a "non-scented" body lotion and a number of other topicals. PTing revealed a number of (previously) relevant reaction, including "Rose centifolia" (5% alc.) and "Rose oil Bulgarian" (2% pet.) essential oil preparations (276). In the An 2005 study, 5 of 422 consecutive patients, i.e., 1.2%, had positive reactions to "Rose oil Bulgarian", tested at 2% concentration (13). Nakayama et al. found 1974 (after (29)) 4 "strong positive" reactions to "Rose oil Bulgarian" (unknown test concentration) in 183 patients. In a study from Alicante, Spain, 86 selected patients were tested with rose oil absolute, yielding 6 positive reactions (48).

Additional information:

ISO 4720:2009 nomenclature: *Rosa x damascena* Mill. and *Rosa sertata* X *Rosa rugosa*. Rose Flower Oil is the volatile oil obtained from the flowers of *Rosa* spp. , rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59362>, last accessed 2009-11-16). "Rose oil, meaning either rose otto (attar of rose, attar of roses) or rose absolute, is the essential oil extracted from the petals of various types of rose. Rose ottos are extracted through steam distillation, while rose absolutes are obtained through solvent extraction or supercritical carbon dioxide extraction, with the absolute being used more commonly in perfumery" (http://en.wikipedia.org/wiki/Rose_oil, last accessed 2009-11-17). There are several more specifically named flower extracts used for masking or perfuming:

- Rosa Alba Flower Extract is an extract obtained from the flowers of the Rose, *Rosa alba* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40969>, last accessed 2009-11-16).
- Rosa Canina Flower Oil is the volatile oil obtained from the flowers of the Hip Rose, *Rosa canina* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59263>, last accessed 2009-11-16).
- Rosa Centifolia Flower Oil is the volatile oil obtained from the flowers of the Cabbage Rose, *Rosa centifolia* (L.), Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79757>, last accessed 2009-11-16).
- Rosa Damascena Flower Oil is the volatile oil obtained from the flowers of the Damask Rose, *Rosa damascena*, Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79760>, last accessed 2009-11-16).
- Rosa Gallica Flower Oil is the volatile oil obtained from the flowers of the French Rose, *Rosa gallica* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=59346>, last accessed 2009-11-16).
- Rosa Moschata Oil is the oil obtained from the Musk Rose, *Rosa moschata*, Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=79761>, last accessed 2009-11-16).
- Rosa Rugosa Flower Oil is the volatile oil obtained from the flowers of the Rose, *Rosa rubiginosa* L., Rosaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=83588>, last accessed 2009-11-16).

Apparently, the *Rosa Damascena* and the *Rosa centifolia* are the species most commonly used for extraction of essential rose oils, the former mostly grown in Bulgaria, Turkey, Russia, India and China, the latter more commonly in Morocco, France and Egypt (276). Main constituents by GC are: citronellol (20-49%), geraniol (6-23%), nerol (3-12%) and phenylethyl alcohol (up to 3.5%) (34).

For Oil of rose (*Rosa x damascena* Miller) an ISO standard exists: ISO 9842:2003.

ROSMARINUS OFFICINALIS FLOWER OILCAS 84604-14-8; EC 283-291-9
(Rosemary, ext.)

"Rosemary Oil"

INCI: ROSMARINUM OFFICINALIS
/ Rosemary, ext.

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to "rosemary" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=3 (3.5%) positive reactions to "rosemary" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Rosmarinus officinalis* L. Rosmarinus Officinalis Flower Oil is an essential oil obtained from the leaves and fresh flowering tops of the Rosemary, *Rosmarinus officinalis* L., Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40978>, last accessed 2010-01-29). Major constituents are: 1,8-cineole (17-55%), alpha-pinene (9-26%), camphor (5-22%) and verbenone [18309-32-5] as traces in North African oils, but between 0.7 and 2.5% in Spanish oils (34). For Oil of rosemary (*Rosmarinus officinalis* L.) an ISO standard exists: ISO 1342:2000.

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

SALVIA spp. HERB OIL

Sage oil

SALVIA OFFICINALIS LAVANDULIFOLIA HERB OILCAS 97952-71-1; EC 308-365-0
(Sage, *Salvia officinalis* *lavandulifolia*, ext. = INCI)**SALVIA LAVANDULIFOLIA HERB OIL**CAS 90106-49-3; EC 290-272-9
(Sage, *Salvia lavandulifolia*, ext. = INCI)**SALVIA SCLAREA FLOWER OIL**CAS 84775-83-7; EC 283-911-8
(Sage, *Salvia sclarea*, ext.) INCI: SALVIA SCLAREA / Sage, *Salvia sclarea*, ext.**SALVIA HISPANICA HERB OIL**CAS 93384-40-8; EC 297-250-8
(Sage, *Salvia hispanica*, ext. = INCI)

Current regulation: /

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "Clary sage", 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to "clary sage" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Salvia officinalis* L. *Salvia Officinalis Lavandulifolia* Herb Oil is an essential oil obtained from the herbs of the Sage, *Salvia officinalis* L. spp. *lavandulifolia*, Lamiaceae, Syn. Dalmatian sage (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41084>, last accessed 2010-01-29).

Salvia Lavandulifolia Herb Oil is an essential oil obtained from the herbs of the Sage, *Salvia lavandulifolia*, Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40987>, last accessed 2010-01-29).

Salvia Sclarea Flower Oil is an essential oil obtained from the flowers and foliage of the Clary Sage, *Salvia sclarea* L., Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41086>, last accessed 2010-01-29).

Salvia Hispanica Herb Oil is an essential oil obtained from the herbs of the Spanish Sage, *Salvia hispanica* L., Lamiaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=40985>, last accessed 2010-01-29).

Clary sage oil is obtained by steam distillation of flowering tops and foliage of cultivated *Salvia sclarea* L. (Lamiaceae). Main constituents are linalyl acetate (56-78%) and linalool (6.5-24%) (34). Dalmatian sage oil is steam distilled from partially dried leaves of *S. officinalis* L. (Lamiaceae). The content by GC is: alpha-thujone (18-43%), beta-thujone (3-8.5%), 1,8-cineole (5.5-13%), camphor (3-8.5%) as main constituents (34). Spanish sage oil does not contain thujone, but mainly camphor (15-36%) and 1,8-cineole (11-30%), and is used mainly in pharmaceutical preparations and technical perfumery (34). For Oil of sage, Spanish (*Salvia lavandulifolia* Vahl) an ISO standard exists: ISO 3526:2005, for Oil of Dalmatian sage (*Salvia officinalis* L.): ISO 9909:1997.

Considering the content of well-known allergenic compounds, this essential oil is regarded as established contact allergen in humans.

SANTALUM ALBUM WOOD OIL

CAS 84787-70-2; EC 284-111-1
(Sandalwood, ext.) INCI:
SANTALUM ALBUM / Sandalwood,
ext.

Sandalwood oil ([East] India)

SANTALUM ALBUM OIL

CAS 8006-87-9; EC / (Oils,
sandalwood)

Sandalwood oil ([East] India)

Current regulation: /

Clinical data:

In the Sugiura 2000 study, 1483 patients with suspected cosmetic dermatitis were PTed with "sandalwood oil" (2% pet.), yielding 0.8% positive reactions (14). In the Frosch 2002 b study, "sandalwood oil (East India)" is mentioned with a CAS # 8015-65-4, which, however, is attributed to AMYRIS BALSAMIFERA BARK OIL, see above. Assuming that this CAS # is erroneous, study results are considered to be valid for *S. album* wood oil, tested at 2% and 10% concentration, yielding 0.4% and 0.9% positive reactions,

respectively (17). Out of 6 of 15 patients with a positive reaction to the higher concentration no clinical relevance was found, compared to 2 of 7 patients positive to the lower concentration (17). The Coimbra 2000 study found in 67 patients with positive reaction to the FM I who were tested with "sandalwood oil" (2% pet.) 6.6% positive reactions (9). In the An 2005 study, 10 of 422 consecutive patients, i.e., 2.4%, had positive reactions to "Santalum album oil" 2% (13). The Goossens 1997 study found 4 of 111 patients positive to "sandalwood oil 10% pet." – all sensitised to other fragrance allergens (23). The Rudzki 1976 study found no positive reaction in 200 patients to "sandalwood", 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=2 (2.3%) positive reactions to "sandalwood" essential oil 2% pet. (27). In 63 patients positive to the FM I, 1 had a positive PT reaction to sandalwood oil, 2% pet., in the Santucci 1987 study (28). Nakayama et al. found 1974 (after (29)) 6 "strong positive" and 8 "weak positive" reactions to "Sandalwood oil" (unknown test concentration) in 183 patients. The IVDK 2010 c study identified 1.3% positive reactions in 3671 consecutively tested patients and 1.8% positive reactions in 1002 patients tested in the context of a special series (30). In a study from Alicante, Spain, 86 selected patients were tested with sandalwood oil, yielding 2 positive reactions (48).

Additional information:

ISO 4720:2009 nomenclature: *Santalum album* L. *Santalum Album* Oil is the volatile oil obtained from the heartwood of the Sandalwood, *Santalum album* L., Santalaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=80209>, last accessed 2009-11-26).

Santalum Album Wood Oil is an essential oil obtained from the wood of the Sandalwood, *Santalum album* L., Santalaceae. It contains 75% santalol isomers (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41092>, last accessed 2009-11-12), typically up to 55% .alpha.-santalol and up to 24% .beta.-santalol (30). East Indian sandalwood oil consists almost exclusively of closely related sesquiterpenoids; by far the main constituents are the alcohols alpha-santalol [115-71-9] (41-55%) and cis-beta-santalol [77-42-9] (16-24%), the latter being mainly responsible for the specific odour (34, 39).

An ISO standard regarding the composition of "*Santalum album* oil" is available: ISO 3518:2002. "Sandalwoods" are labelled as *Amyris balsamifera*, *Eremophila mitchelli*, *Fusanus acuminatus* (= *Santalum acuminatum*), *Santalum album*, *S. austrocaledonicum*, *S. latifolium*, *S. spicatum* and *S. yasi*. The majority of currently available trade oils, reportedly from *S. album*, contained approximately 50-70% santalols (Z-alpha and Z-beta), as analysed with gas chromatography-mass spectrometry (GC-MS) (277). A review on the toxicological properties of "*Santalum album* oil" is available (278).

AMYRIS BALSAMIFERA BARK OIL (*Sandalwood oil (Caribbean)*), CAS 8015-65-4; EC / (Oils, amyris) / 90320-49-3; EC 90320-49-3 (*Amyris balsamifera*, ext. = INCI name) is used as a cheap substitute for East Indian Sandalwood in perfumes and cosmetics. Originally cultivated primarily in Haiti where it was known as 'candle wood' and used as a torch by locals due to the tree's high oil content (<http://www.amphora-retail.com/sandalwood-amyris-essential-10ml-p-107.html>, last accessed 2009-11-12). The major components are sesquiterpenoids such as valerianol, elemol, β -eudesmol and epi-gamma-eudesmol (39). For Oil of amyris (*Amyris balsamifera* L.) an ISO standard exists: ISO 3525:2008. *Amyris Balsamifera* Bark Oil is the volatile oil distilled from the bark of the tree, *Amyris balsamifera*, Rutaceae (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=74455>, last accessed 2009-11-12).

SANTALUM SPICATA WOOD OILCAS 8024-35-9; EC 296-618-5
(Sandalwood oil, Western
Australia)*Sandalwood oil (Australia)*

Current regulation: /

Clinical data:

In clinical studies, mostly *S. album* wood oil had been used (see above); in a number of studies this is not clear.

Additional information:

ISO 4720:2009 nomenclature: *Santalum spicatum* (R.Br.) A. DC, syn. *Eucarya spicata* (R.Br.) Sprag & Summ. For Oil of Australian sandalwood (*Santalum spicatum* (R.Br.) A.DC.) an ISO standard exists: ISO 22769:2009. Santalum Spicata Wood Oil is an essential oil obtained from the wood of the Australian Sandalwood, *Santalum spicata*, Santalaceae. It contains 75% santalols and 10% farnesol (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41093>, last accessed 2009-11-12). This oil also contains santalols as main constituents but differs somewhat in the remaining composition. Today, it makes up a considerable part of the sandalwood oil market (34).

Considering the content of well-known allergenic compounds (santalols), this essential oil is regarded as established contact allergen in humans.

TAGETES PATULA FLOWER OILCAS 91722-29-1; EC 294-431-3
(*Tagetes patula*, ext. = INCI)*"Marigold Oil; Tagetes Oil"*

Current regulation: /

Clinical data:

In an aromatherapist, an essential oil solvent-extracted from *Tagetes patula*, patch tested at 1.5% in grapeseed oil (vehicle negative, 7 controls negative to essential oils) resulted in a +++ reaction, in accordance with a work-related bilateral hand dermatitis (217).

Additional information:

Tagetes Patula Flower Oil is an essential oil obtained by hydrodistillation of the flowers of the *Tagetes*, *Tagetes patula* L., *Compositae*. It contains mainly D-limonene, ocimene, 2,6-dimethyloct-7-en-4-one (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41506>, last accessed 2010-01-28). According to Surburg/Panten, tagetes oil is steam distilled from the flowering plants of *Tagetes minuta* L. (*T. glandulifera* Schrank., *Asteraceae*). Main components comprise cis-ocimene, dihydrotagetone, tagetone, and cis- and trans-ocimenone (34, 39).

THYMUS spp. HERB OIL

THYMUS VULGARIS HERB OIL

CAS 84929-51-1, 8007-46-3; EC 284-535-7 (Thyme, Thymus vulgaris, ext.)

"Thyme oil"

INCI: THYMUS VULGARIS / Thyme, Thymus vulgaris, ext.

Current regulation: /

Clinical data:

The Rudzki 1976 study found no positive reaction in 200 patients to "thyme" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=4 (4.6%) positive reactions to "thyme" essential oil 2% pet. (27). In 63 patients positive to the FM I, none had a positive PT reaction to thymol, 1% pet., in the Santucci 1987 study (28).

Additional information:

ISO 4720:2009 nomenclature: *Thymus vulgaris* L. *Thymus vulgaris* Herb Oil is an essential oil obtained from the herbs of the Thyme, *Thymus vulgaris* L., Lamiaceae. It contains 20-40% thymol and carvacrol, cymene, pinene, linalool, bornyl acetate (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41133>, last accessed 2010-01-29).

Other species are used for extraction, e.g., *Thymus Mastichina* (CAS 84837-14-9), *Thymus Serpillum* (CAS 84776-98-7), *Thymus Zygis* (CAS 85085-75-2), according to CosIng. The main constituent is thymol (37-56%) (34). For Oil of thyme containing thymol, Spanish type [*Thymus zygis* (Loefl.) L.] an ISO standard exists: ISO 14715:2010, for Oil of Spanish wild marjoram (*Thymus mastichina* L.): ISO 4728:2003.

TURPENTINE (oil)

CAS 8006-64-2 / 9005-90-7 / 8052-14-0; EC 232-350-7 / 232-688-5 / -

Current regulation: III/124 ; III/125 ; III/126

Clinical data:

Oil of turpentine has been patch tested in a number of baseline series, i.e., in consecutive patients, although not included in the European Baseline series.

In a series of 24 patients with occupational contact dermatitis from the pottery industry, Lear at al. found 14 to be sensitised to "Indonesian oil of turpentine" and 8 to alpha-pinene (190)

Table 3.2.2 – 2: Overview of results with **Oil of turpentine** in patients patch tested for suspected allergic contact dermatitis. If not given in the publication, the confidence interval (CI) was calculated from the absolute numbers by the SCCS.

Country	Population	Years	No. tested	Crude % positive (95% CI) §
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Opinion on fragrance allergens in cosmetic products

Lisbon, Portugal (189); virtually no .delta.-3-carene	Consecutive patients	1979-1983	4316	2.3 (1.9 – 2.8) § %
Birmingham, UK (190)	Potters with occup. dermatitis	6 months; prior to 1996	24	14 / 24 pos. to "Indonesian turpentine"
Austria/Germany (IVDK) (279)	Consecutive patients	1992-1995	27658	0.47 (0.39 – 0.55) § %
Austria/Germany (IVDK) (280)	Consecutive patients	1996-2002	59478	Annual prevalences 1.6 to 4.4 %
Augsburg/Germany (281)	Population sample	1998	1141	1.2% (on population level!)
Europe (ESSCA) (273)	Consecutive patients	2002/03	3767	1.6 %
Austria/Germany/Switzerland (IVDK) (7)	Consecutive patients	2005-2008	37163	1.8 %

Additional information:

ISO 4720:2009 nomenclature: *Pinus pinaster* Aiton and *Pinus massoniana* Lamb. Turpentine, oil: Any of the volatile predominately terpenic fractions or distillates resulting from the solvent extraction of, gum collection from, or pulping of softwoods. Turpentine is a mixture of terpene hydrocarbons obtained from various species of *Pinus* http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details_v2&id=41521

The composition of oil of turpentine varies with its origin, in particular, the content of .delta.-3-carene, one of its main allergenic compounds (189, 279). Similarly, the peroxide degree may vary. The main constituents are .alpha.-pinene (50-72%), .beta.-pinene (6-15%), carenes (< 0.1-17%), camphene (up to 1%), dipentene (0.5-5%), along with a number of other substances (279).

It is a "top 200" substance and classified as R43 (IFRA, pers. comm.2010)

Verbena absolute (Lippia citriodora Kunth.)

CAS 8024-12-2, 84961-67-1; EC /)

Current regulation: Annex III, part 1, n° 206

Clinical data: /

Additional information:

ISO 4720:2009 nomenclature: *Aloysia citriodora* Palau syn. *Lippia citriodora* Kunth syn. *Aloysia triphylla* (L' Hér.) Kuntze. An older RIFM review is available citing several positive human maximisation studies both with "Verbena absolute" and "Verbena oil" (128).

VETIVERIA ZIZANOIDES ROOT OIL

CAS 8016-96-4; EC / (Oils, vetiver) / 84238-29-9; EC 282-490-8 (Vetiveria zizanioides, ext. = INCI)

"Vetiver oil; khas khas oil"

Current regulation: ...

Clinical data:

The Rudzki 1976 study found 1 positive reaction in 200 patients to "vetiver" essential oil, 2% pet. (26). The later Rudzki 1986 study in 86 FM I positive patients found n=9 (10.4%) positive reactions to "vetiver" essential oil 2% pet. (27).

Additional information:

ISO 4720:2009 nomenclature: *Vetiveria zizanioides* (L.) Nash. *Vetiveria Zizanioides* Root Oil is an essential oil distilled from the dried roots of the grass *Vetiveria zizanioides* (L.) Nash *Poaceae* (<http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.details&id=41293>, last accessed 2010-01-29). Vetiver oil has a high sesquiterpene content. The ketones alpha-vetivone [15764-04-2] (6-12%) and beta-vetivone [18444-79-6] (4-10%), which usually form more than 10% of the oil, as well as khusimol [16223-63-5] (24-36%) and isovelencenol [22387-74-2] (12-24%) are the main constituents (in Bourbon oil, i.e., from Réunion) (34). For Oil of vetiver (*Vetiveria zizanioides* (L.) Nash) an ISO standard exists: ISO 4716:2002.

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Annex II - Animal Data

Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Substance		EC3 value *							Comment (deviation from OECD 429 etc)	Reference
INCI name (other name)	CAS no.	Vehicle (AOO=acetone:olive oil; DEP=diethyl phthalate; DMF=dimethyl formamide; DMSO=dimethyl sulphoxide; EtOH=ethanol; MEK=methyl ethyl ketone)	Conc. in vehicle (% generally w/v)	No. animals per dose group	%	µg/cm ²	M	lowest for the substance (%)		
<i>Allyl phenoxyacetate</i>	7493-74-5	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	4	3.1	775	0.16	3.1		RIFM, 2007a
Amyl cinnamal	122-40-7	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	7.6	1900	0.38	7.6		RIFM, 2006a
Amyl cinnamal	122-40-7	4:1 AOO	-	4	10.6	2650	0.52		Elahi gives ref to Basketter et al 1999, but no data on the substance is found. It is not known if Elahi, Aptula and Roberts quote the same experiment	Elahi et al., 2004

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Amyl cinnamal	122-40-7	-	-	-	11	2750	0.54		Aptula gives ref to Kimber et al 2003, but no LLNA data on the substance is found. It is not known if Elahi, Aptula and Roberts quote the same experiment; original reference is not given.	Aptula et al., 2007
Amyl cinnamal	122-40-7	-	-	-	11	2750	0.54		Original ref not given.	Roberts et al., 2007
Amylcinnamyl alcohol	101-85-9	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	> 25	>6250	>1.22	> 25	Should have been tested at higher concentrations	RIFM, 2004a
Anise alcohol	105-13-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	5.9	1475	0.43	5.9		RIFM, 2005a
Benzaldehyde	100-52-7	-	-	-	-	-	-		No data in the ref	Roberts et al., 2007
Benzaldehyde	100-52-7	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2003
Benzyl alcohol	100-51-6	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	> 50	>12500	>4.62	> 50	Should have been tested at higher concentrations	RIFM, 2005b

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Benzyl benzoate	120-51-4	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	> 50	>12500	>2.36	> 50	Should have been tested at higher concentrations	RIFM, 2005c
Benzyl cinnamate	103-41-3	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	18.4	4600	0.77	18.4		RIFM, 2005d
Benzyl salicylate	118-58-1	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	2.9	725	0.13	2.9		RIFM, 2005e
<i>p</i> -tert-Butyl-dihydrocinnamaldehyde	18127-01-0	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	4.3	1075	0.23	4.3		RIFM, 2007b
Butylphenyl methylpropional (BMHCA)	80-54-6	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	2.9	725	0.14	2.9		RIFM, 2001a
Butylphenyl methylpropional (BMHCA)	80-54-6	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	4.1	1025	0.20			RIFM, 2001b
Butylphenyl methylpropional (BMHCA)	80-54-6	1:3 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	13.9	3475	0.68			RIFM, 2001c
Butylphenyl methylpropional (BMHCA)	80-54-6	1:3 DEP:EtOH	0.3, 1.0, 3.0, 10.0, 30.0	4	8.8	2200	0.43			RIFM, 2001d
Butylphenyl methylpropional (BMHCA)	80-54-6	4:1 AOO	1.0, 2.5, 5.0, 10.0, 25.0	4	16.8	4200	0.82			RIFM, 2001e

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Butylphenyl methylpropional (BMHCA)	80-54-6	4:1 AOO	1, 2.5, 10, 25, 50	4	18.7	4675	0.92		Basketter et al., 2001
Camellia sinensis leaf Tea Leaf Absolute	84650-60-2	DMF	0.5, 1.0, 2.5, 5.0, 10.0	4	> 5.0	>1250	N/a	> 5.0	Should have been tested at higher concentrations RIFM, 2005m
Cananga odorata leaf / flower oil Ylang Ylang Extra	8006-81-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	4	6.8	1700	N/a	6.8	RIFM, 2007f
Carvone	6485-40-1	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	10.7	2675	0.71		RIFM, 2007c
Carvone	6485-40-1	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	5.7	1425	0.38	5.7	RIFM, 2007d
Carvone	6485-40-1	4:1 AOO	6.0, 12, 20	4	13	3250	0.86		Nilsson et al., 2005
Cinnamal	104-55-2	3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.2	50	0.015	0.2	RIFM, 2003a
Cinnamal	104-55-2	0.1% α -tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.2	50	0.015		RIFM, 2003b
Cinnamal	104-55-2	2.0% α -tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.6	150	0.045		RIFM, 2003c
Cinnamal	104-55-2	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1	0.1, 0.3, 1.0, 3.0, 10.0	4	0.7	175	0.053		RIFM, 2003d

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

EtOH:DEP

Cinnamal	104-55-2	0.1% Trolox C in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.7	175	0.053		RIFM, 2003e
Cinnamal	104-55-2	2.0% α -tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.8	200	0.060		RIFM, 2003f
Cinnamal	104-55-2	3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	0.9	225	0.068		RIFM, 2003g
Cinnamal	104-55-2	0.1% α -tocopherol in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.1	275	0.083		RIFM, 2003h
Cinnamal	104-55-2	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.3	325	0.098		RIFM, 2003i
Cinnamal	104-55-2	0.1% Trolox C in 3:1 EtOH:DEP	0.1, 0.3, 1.0, 3.0, 10.0	4	1.4	350	0.11		RIFM, 2003j
Cinnamal	104-55-2	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Cinnamal	104-55-2	4:1 AOO	0.5, 1, 2.5, 5, 10	4	3.1	775	0.23		Basketter et al., 2001
Cinnamal	104-55-2	4:1 AOO	-	4	1.3	325	0.10		Elahi et al., 2004
Cinnamal	104-55-2	4:1 AOO	1, 2.5	-	1.4	348	0.11	Too few concentrations tested; few details given in ref	Smith and Hotchkiss, 2001
Cinnamal	104-55-2	4:1 AOO	1.0, 2.5, 5.0, 10.0, 25.0	4	1.7	425	0.13		Wright et al., 1995

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Cinnamal	104-55-2	MEK	1.0, 2.5, 5.0, 10.0, 25.0	4	1.1	275	0.083		Wright et al., 1996
Cinnamal	104-55-2	DMF	0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0	4	0.5	125	0.038		Wright et al., 1997
Cinnamal	104-55-2	propylene glycol	1.0, 2.5, 5.0, 10.0, 25.0	4	1.4	350	0.11		Wright et al., 1998
Cinnamal	104-55-2	DMSO	0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0	4	0.9	225	0.068		Wright et al., 1999
Cinnamal	104-55-2	90:10 EtOH:water	1.0, 2.5, 5.0, 10.0, 25.0	4	1.6	400	0.12		Wright et al., 2000
Cinnamal	104-55-2	50:50 EtOH:water	1.0, 2.5, 5.0, 10.0, 25.0	4	1.2	300	0.091		Wright et al., 2001
Cinnamyl alcohol	104-54-1	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Cinnamyl nitrile	1885-38-7	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	> 10	>2500	>0.77	> 10	Report: systemic toxicity at 25% and 50%. Should have been tested at higher concentrations RIFM, 2005f
Citral	5392-40-5	1:3 EtOH:DEP	0.4, 2.0, 4.0, 8.0, 20.0	4	1.2	300	0.079	1.2	RIFM, 2004b
Citral	5392-40-5	0.1% α -tocopherol in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	1.5	375	0.099		RIFM, 2003k

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Citral	5392-40-5	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	2.1	525	0.14		RIFM, 2003l
Citral	5392-40-5	0.1% Trolox C in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	3.7	925	0.24		RIFM, 2003m
Citral	5392-40-5	3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	4.6	1150	0.30		RIFM, 2003n
Citral	5392-40-5	0.3% antioxidant mix (equal parts BHT, tocopherol and eugenol) in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	4.6	1150	0.30		RIFM, 2003o
Citral	5392-40-5	3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	5.3	1325	0.35		RIFM, 2003p
Citral	5392-40-5	0.1% Trolox C in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	5.8	1400	0.38		RIFM, 2003q
Citral	5392-40-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	6.3	1575	0.41		RIFM, 2003r
Citral	5392-40-5	0.1% α -tocopherol in 3:1 EtOH:DEP	0.3, 1.0, 3.0, 10.0, 30.0	4	6.8	1700	0.44		RIFM, 2003s
Citral	5392-40-5	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Citronellol	106-22-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	43.5	10875	2.78	43.5	RIFM, 2004c

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Coumarin	91-64-5	DMF	10, 25, 50	4	>50	>12500	>3.42	>50	Should have been tested at higher concentrations	Vocanson et al., 2006
<i>Dibenzyl ether</i>	103-50-4	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	6.3	1575	0.32	6.3		RIFM, 2007e
Eugenol	97-53-0	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	5.3	1325	0.32	5.3		RIFM, 2001f
Eugenol	97-53-0	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	10.5	2625	0.64			RIFM, 2001g
Eugenol	97-53-0	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	10.7	2675	0.65			RIFM, 2001h
Eugenol	97-53-0	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	15.1	3775	0.92			RIFM, 2001i
Eugenol	97-53-0	4:1 AOO	2.5, 5.0, 10.0, 25.0, 50.0	-	11.9	2975	0.72			Basketter et al., 1999
Eugenol	97-53-0	-	-	-	-	-	-		No data in the ref (poster abstract)	Basketter et al., 2003
Evernia furfuracea extract <i>Treemoss absolute</i>	90028-67-4	1:3 EtOH:DEP	5.0, 10.0, 20	4	> 20	>5000	N/a	> 20	Should have been tested at higher concentrations	RIFM, 2004k
Evernia furfuracea extract <i>Treemoss absolute</i>	90028-67-4	1:3 EtOH:DEP	10.0, 25.0	4	> 25	>6250	N/a		Too few concentrations tested	RIFM, 2004d

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Evernia prunastri extract <i>Oakmoss</i>	90028-68-5	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	3.88	970	N/a	3.88		RIFM, 2004j
Farnesol	4602-84-0	4:1 AOO	5.0, 10.0, 25.0	4	5.5	1375	0.25		Should also have been tested at lower concentrations	RIFM, 2004d
Farnesol	4602-84-0	4:1 AOO	5.0, 10.0, 25.0	4	4.1	1025	0.18	4.1	Should also have been tested at lower concentrations	RIFM, 2004d
Geraniol	106-24-1	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	5.6	1400	0.36	5.6		RIFM, 2001j
Geraniol	106-24-1	3:1 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	11.4	2850	0.74			RIFM, 2003t
Geraniol	106-24-1	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	11.8	2950	0.76			RIFM, 2001k
Geraniol	106-24-1	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	20.4	5100	1.32			RIFM, 2001l
Geraniol	106-24-1	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	25.8	6450	1.67			RIFM, 2001m
Geraniol	106-24-1	-	-	-	26	6500	1.69			Roberts et al., 2007
<i>trans-2-Hexenal</i>	6728-26-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5, 10	4	2.6	650	0.26	2.6		RIFM, 2005g
<i>trans-2-Hexenal</i>	6728-26-3	-	-	-	5.5	1375	0.56			Roberts et al., 2007

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Hexyl cinnamal	101-86-0	generally 4:1 AOO		-O162	5.3-14.7	1325-3675	0.25-0.68	5.3	"numerous accounts in the literature"
2-Hexylidene cyclopentanone	17373-89-6	1:3 EtOH:DEP	0.1, 0.5, 1.0, 2.5, 5.0	5	2.4	600	0.14	2.4	RIFM, 2008a
Hexyl salicylate	6259-76-3	1:3 EtOH:DEP	0.05, 0.25, 0.5, 1.0, 2.5	4	0.18	45	0.008	0.18	RIFM, 2006b
Hydroxycitronellal	107-75-5	1:3 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	19.3	4825	1.12	19.3	RIFM, 2001n
Hydroxycitronellal	107-75-5	DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	19.7	4925	1.14		RIFM, 2001o
Hydroxycitronellal	107-75-5	3:1 EtOH:DEP	1.0, 3.0, 10.0, 30.0, 50.0	4	22.2	5550	1.29		RIFM, 2001p
Hydroxycitronellal	107-75-5	EtOH	1.0, 3.0, 10.0, 30.0, 50.0	4	26.4	6600	1.53		RIFM, 2001q
Hydroxycitronellal	107-75-5	AOO	25, 50, 100	-	-	-	-	EC3 value not given	Ashby et al., 1995
Hydroxycitronellal	107-75-5	4:1 AOO	2.5, 5, 10, 25, 50	4	33.0	8250	1.92		Basketter et al., 2001
Hydroxycitronellal	107-75-5	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Hydroxycitronellal	107-75-5	-	-	-	25.25	6313	1.47		Estrada et al., 2003

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Hydroxycitronellal	107-75-5	4:1 AOO	10, 25	-	23	5750	1.34		Too few concentrations tested; few details given in ref	Smith and Hotchkiss, 2001
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	31906-04-4	4:1 AOO	1.0, 2.5, 5, 10, 25, 50	4	17.1	4275	0.81	17.1		RIFM, 2001r
<i>p</i> -Isobutyl- α -methyl hydrocinnamaldehyde	6658-48-6	70% EtOH	10.0, 25.0, 50.0, 100.0	4	9.5	2375	0.46	9.5	Should also have been tested at lower concentrations	RIFM, 2001w
<i>Isocyclocitral</i>	1335-66-6	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	4	7.3	1825	0.48	7.3		RIFM, 2006c
<i>Isocyclogeraniol</i>	68527-77-5	1:3 EtOH:DEP	5.0, 10.0, 25.0, 50.0	4	> 25	>6250	>1.62	> 25	Should have been tested at higher concentrations	RIFM, 2005h
Isoeugenol	97-54-1	4:1 AOO	0.5, 5.0	6	0.54	145	0.033	0.54	Too few concentrations tested	RIFM, 2001s
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.6	150	0.037			RIFM, 2002a
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.76	191	0.046			RIFM, 2002b
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	0.79	199	0.048			RIFM, 2002c

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Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.19	296	0.072	RIFM, 2001t
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.28	320	0.078	RIFM, 2004e
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	6	1.54	385	0.094	RIFM, 2001u
Isoeugenol	97-54-1	4:1 AOO	0.5, 1.0, 5.0	5	1.95	488	0.119	RIFM, 2001v
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0		3.3	825	0.20	Basketter et al., 1999
Isoeugenol	97-54-1	-	-	-	-	-	-	No data in the ref (poster abstract) Basketter et al., 2002
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.3	325	0.079	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	3.3	825	0.20	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.8	450	0.11	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	3.1	775	0.19	Loveless et al., 1996
Isoeugenol	97-54-1	4:1 AOO	0.25, 0.5, 1.0, 2.5, 5.0	4 or 5	1.6	400	0.097	Loveless et al., 1996
Isoeugenol	97-54-1	AOO	0.5, 1.0, 2.5, 5.0, 10.0	4	1.0	250	0.061	Wright et al., 2001
Isoeugenol	97-54-1	MEK	0.5, 1.0, 2.5, 5.0, 10.0	4	1.0	250	0.061	Wright et al., 2001
Isoeugenol	97-54-1	DMF	0.5, 1.0, 2.5, 5.0, 10.0	4	1.4	350	0.085	Wright et al., 2001

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Isoeugenol	97-54-1	propylene glycol	0.5, 1.0, 2.5, 5.0, 10.0	4	2.5	625	0.15			Wright et al., 2001
Isoeugenol	97-54-1	DMSO	0.5, 1.0, 2.5, 5.0, 10.0	4	0.9	225	0.055			Wright et al., 2001
Isoeugenol	97-54-1	90:10 EtOH:water	0.5, 1.0, 2.5, 5.0, 10.0	4	1.8	450	0.11			Wright et al., 2001
Isoeugenol	97-54-1	50:50 EtOH:water	0.5, 1.0, 2.5, 5.0, 10.0	4	4.9	1225	0.30			Wright et al., 2001
Jasmine absolute (Grandiflorum)	8022-96-6	1:3 EtOH:DEP	1.0, 2.5, 5.0, 10.0, 25.0	4	5.9	1475	N/a	5.9		RIFM, 2006d
Jasminum Sambac Flower CERA / Extract / Water	91770-14-8	1:3 EtOH:DEP	10.0, 25.0, 50.0, 75.0, 100.0	4	35.4	9100	N/a	35.4		RIFM, 2006e
d-Limonene**	5989-27-5	EtOH	10.0, 20.0, 50.0, 75.0, 100.0	4	< 10	< 250	<0.73	< 10	Should also have been tested at lower concentrations	RIFM, 2004l
d-Limonene**	5989-27-5	3:1 EtOH:DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	22.0	5500	1.61			RIFM, 2004m
d-Limonene**	5989-27-5	1:3 EtOH:DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	38.0	9500	2.79			RIFM, 2004n
d-Limonene**	5989-27-5	DEP	10.0, 20.0, 50.0, 75.0, 100.0	4	63.0	15.75	4.62			RIFM, 2004o
d-Limonene**	5989-27-5	4:1 AOO	25, 50, 100	4	68.5	17125	5.03			Warbrick et al., 2001

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Annex II . Local lymph node assay (LLNA) data on 59 fragrance substances, based on a summary report submitted by the Research Institute for Fragrance Materials, Inc. (RIFM, 2009)

Linalool**	78-70-6	-	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2002
Menthadiene-7-methyl formate	68683- 20-5	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	> 10	> 2500	>0.51	> 10	Should have been tested at higher concentrations	RIFM, 2008c
4-Methoxy- α -methyl benzenopropanal	5462- 06-6	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	23.6	5900	1.32	23.63		RIFM, 2004f
α -Methyl cinnamic aldehyde	101-39- 3	-	-	-	4.5	1125	0.31	4.5		Roberts et al., 2007
Methylenedioxyphenyl methylpropanal	1205- 17-0	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	16.4	4100	0.85	16.4		RIFM, 2005i
6-Methyl-3,5-heptadien-2- one	1604- 28-0	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	> 5	> 1250	>0.40	> 5	Should have been tested at higher concentrations	RIFM, 2008d
α -iso-Methylionone	127-51- 5	1:3 EtOH:DEP	10.0, 25.0, 50.0, 75.0, 100.0	4	21.8	5450	1.06	21.8		RIFM, 2005j
Methyl octine carbonate	111-80- 8	-	-	-	2.5	635	0.15	2.5		Roberts et al., 2007

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Methyl 2-octynoate	111-12-6	1:3 EtOH:DEP	0.5, 1.0, 2.0, 5.0, 10.0	4	< 0.5	< 125	<0.032	< 0.5	Should also have been tested at lower concentrations	RIFM, 2005k
2-Methoxy-4-methylphenol	93-51-6	-	-	-	5.8	1450	0.42	5.8		Roberts et al., 2007
1-Octen-3-yl acetate	2442-10-6	1:3 EtOH:DEP	7.5, 15.0, 30.0	5	> 30	> 7500	>1.76	> 30	Should have been tested at higher concentrations	RIFM, 2004g
Perillaldehyde <i>p</i> -Mentha-1,8-dien-7-al	2111-75-3	1:3 EtOH:DEP	0.5, 1.0, 2.5, 5.0, 10.0	5	9.3	2325	0.62			RIFM, 2008b
Perillaldehyde <i>p</i> -Mentha-1,8-dien-7-al	2111-75-3	-	-	-	8.1	2025	0.54	8.1		Roberts et al., 2007
Balsam oil, Peru (<i>Myroxylon pereirae</i> Klotzsch)	8007-00-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	3.95	987	N/a	3.95		RIFM, 2004h
Peru balsam absolute	8007-00-9	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	2.5	625	N/a	2.5		RIFM, 2004i
Peru balsam absolute	8007-00-9	1:3 EtOH:DEP	0.5, 1.0, 2.5	4	>2.5	>625	N/a			RIFM, 2004i
Phenylacetaldehyde	122-78-1	4:1 AOO	2.5, 5, 10, 25, 50	4	3.0	750	0.25	3.0		Basketter et al., 2001

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<i>Phenylacetaldehyde</i>	122-78-1	-	-	-	-	-	-	-	No data in the ref (poster abstract)	Basketter et al., 2003
<i>3-Propylidenephthalide</i>	17369-59-4	4:1 AOO	5, 10, 20	4 or 5	3.7	925	0.21	3.7	Should also have been tested at lower concentrations	Gerberick et al., 2004
Tetramethyl acetyloctahydronaphthalenes (OTNE)	54464-57-2	1:3 EtOH:DEP	2.5, 5.0, 10.0, 25.0, 50.0	4	25.14	6285	1.07	25.14		RIFM, 2005i
Trimethylbenzenepropanol <i>Majantol</i>	103694-68-4	4:1 AOO	3.0, 10.0, 30.0	4	~30	~7500	~1.68	30	Should have been tested at higher concentrations	RIFM, 2002d
Vanillin	121-33-5	4:1 AOO	2.5, 5, 10, 25, 50	4	>50.0	>1250	>3.3	>50.0		Basketter et al., 2001

* source of EC3 value value: % given in the RIFM report or references; µg/cm2 given in the RIFM report and RIFM poster; M calculated by SCCS working group

**material with low levels of oxidation according to RIFM, 2009

- = no data given; A216

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Annex III - Tabular summary of dose-elicitation studies in sensitised patients**Contents**

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Chloroatranol

Chloroatranol (allergen in oak moss absolute: <i>Evernia prunastri</i>) (1)	
Design	blinded, randomised with regard to doses and controlled
Test subjects	13 patients previously identified as sensitized to chloroatranol and oak moss absolute
Controls	10 healthy controls
Substance	Purity: >99%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	200 ppm to 0.0063 ppm (10 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction
ROAT	volar aspect of forearms
area	3 x 3 cm ²
applications/day	two
dose	chloroatranol in ethanol: Step 1: 5 ppm Step 2: 25 ppm
dose/application/cm ²	step 1: 0.025 µg step2: 0.125 µg
control substance	ethanol
definition of positive	erythema in at least 25% and at least one papule
period	two weeks for each step
Results	
PT ED10% (95% CI)	0.013 (0.002-0.03) ppm =0.0004 µg/cm ²
PT ED50% (95% CI)	0.15 (0.077-0.295) ppm =0.0045 µg/cm ²
PT no effect level (observed)	/
ROAT	Cumulative responses
Step 1 (5 ppm)	12/13 (92%)
Step 2 (25 ppm)	13/13 (100%)
Controls	Negative
Other information	None relevant

In a subsequent study chloroatranol and atranol, both ingredients in *Evernia prunastri*, were tested in equimolar concentrations in serial dilution in 10 eczema patients with known sensitization to chloroatranol and oak moss. A positive response was defined as any degree of reaction. Ethanol was included as the control and gave no response. No use tests were done and no control subjects included.

Results: All patients reacted to the highest concentrations of the two substances. For both substances there was a significant dose-dependence and the estimated difference in elicitation potency of chloroatranol relative to atranol was 217%. The dose-response curve is seen in figure 1 below (2).

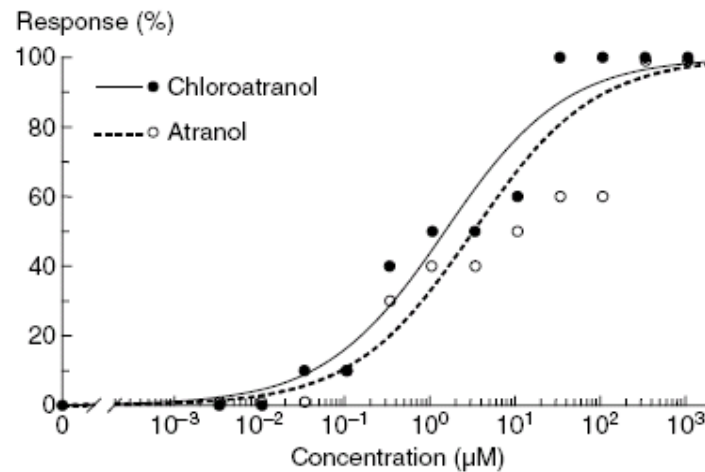


Fig. 1. Observed response rates and fitted parallel logistic dose-response curves for atranol and chloroatranol in equimolar concentrations at patch testing. The response was dichotomized and any reaction other than zero was classified as positive.

Cinnamal

Cinnamal (3)	
Design	blinded, randomised and controlled
Test subjects	18 patients with a positive patch test to cinnamal and additional 4 with a doubtful response
Controls	20 healthy controls
Substance	Purity: >98%
Patch test	20 mg solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.01% (7 steps)
-control/vehicle	petrolatum
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of responses
ROAT	outer aspect of upper arm
area	5 x 5 cm ²
applications/day	two with atomizer pump
dose	Step 1: 0.02% Step 2: 0.1% Step 3: 0.8%
dose/application/cm ²	Not given
control substance	ethanol
definition of positive	The response was classified as positive no matter the degree of reaction.
period	two weeks for each step; total maximum 6 weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	0.24% = 96 µg/cm ² (calculated from the data in the paper)
PT no effect level(observed)	0.01 % in pet. = 0.4 µg/cm ²
ROAT	Cumulative responses
Step 1 (0.02%)	0/18
Step 2 (0.1%)	8/18 (44 %)
Step 3 (0.8%)	13/18 (72 %)
Controls	No eczema reactions were seen
Other information	2 patients and 2 controls developed immediate reactions to the cinnamal solution

Cinnamal (4)	
Design	blinded, randomised doses and controlled
Test subjects	17 patients with a positive patch test to cinnamal (8 patients in part 1 and 9 in part two)
Controls	20 controls (non-sensitised dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2 % to 0.00006 % (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting a + reaction
ROAT	Axilla
area	10 x 10 cm ² (estimated)
applications/day	two with roll on deodorant (89-700 mg per application of solution) average cases: 263 mg/application controls: only range given
dose	Part one: Step 1: 0.032% Step 2: 0.1% Step: 0.32% Part two: Step 1: 0.01% Step 2: 0.032% Step 3: 0.1%
dose/application/cm ²	Part two estimated: step one: 0.26 µg; step two: 0.84 µg; 2.63 µg
control substance	Deodorant matrix
definition of positive	eczematous reaction covering at least 25% of test area
period	Part one: one week with each concentration: maximum three weeks Part two: two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level(observed)	0.002%
ROAT	Cumulative responses
Step 1 (0.01)	2/9 (22%)
Step 2 (0.032)	6/9 (67%)
Step 3 (0.1)	8/9 (88%)
Controls	No reactions were seen
Other information	Only reactions seen to the cinnamal-containing deodorants at ROAT, difference to matrix axilla ($p<0.001$) and all control

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	persons negative ($p < 0.001$)
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Hydroxycitronellal

Hydroxycitronellal (5)	
Design	blinded, randomised doses and controlled
Test subjects	7 patients with a positive patch test to hydroxycitronellal
Controls	7 controls (non-sensitised dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	4% to 0.00006% (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting + reaction
ROAT	Axilla
area	10 x 10 cm ² (estimated)
applications/day	two with roll on deodorant (172-591 per application of solution) average cases: 294 mg/application controls: only range given
dose	Step 1: 0.032% Step 2: 0.1% Step: 0.32%
dose/application/cm ²	Estimated: step 1: 0.94 µg; step 2: 2.94 µg; step 3: 9.40 µg
control substance	Deodorant matrix
definition of positive	eczematous reaction covering at least 25% of test area
period	two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level(observed)	<0.00012 %
ROAT	Cumulative responses
Step 1 (0.032)	4/7 (57%)
Step 2 (0.1)	5/7 (71%)
Step 3 (0.32)	7/7 (100%)
Controls	No reactions were seen
Other information	Reactions were only seen to the hydroxycitronellal-containing deodorant at ROAT, difference to matrix treated axilla ($p<0.001$) and all control persons negative ($p<0.001$)

Hydroxycitronellal (6)	
Design	double blinded, randomised
Test subjects	13 patients with a positive patch test to hydroxycitronellal
Controls	/
Substance	purity: unknown
Patch test	confirmatory
-dilution steps	
-control/vehicle	
-definition of threshold	
ROAT	finger immersion in fragrance solution in 10% ethanol
area	/
applications/day	Once per day for 10 min
dose	Step 1: 10 ppm Step 2: 250 ppm
dose/application/cm ²	Not applicable
control substance	10% alcohol
definition of positive	clinical grading scale and laser doppler comparison between active and control
period	two weeks with each concentration: maximum four weeks
Results	
PT ED10% (95% CI)	Not relevant
PT ED50% (95% CI)	Not relevant
PT no effect level(observed)	Not relevant
ROAT	Cumulative responses
Step 1 (10 ppm)	1/13
Step 2 (250 ppm)	5/13
Vehicle control	4/13
Other information	No difference between active substance and control application was found.

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC)

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) (7)	
Design	blinded, randomised and controlled
Test subjects	18 patients with a positive patch test to HICC
Controls	7 healthy controls
Substance	Purity: >99%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	6% to 0.0006%
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions
ROAT	volar aspect of lower arm
area	3 x 3 cm ²
applications/day	two with droplet bottle (theoretical:30 mg per application of solution)
dose	Step 1: 0.5% Step 2: 3%
µg/application/cm ²	Step 1: 15.3 (3.4-22.2) Step 2: 126.2 (40.5-226.2)
control substance	ethanol
definition of positive	erythema in at least 25% and at least one papule
period	two weeks for each step; total maximum 4 weeks
Results	
PT ED10% (95% CI)	0.9 µg/cm ² 29 (7-69) ppm
PT ED50% (95% CI)	20 µg/cm ² 662 (350-1250)ppm
PT no effect level (observed)	/
ROAT	Cumulative responses
Step 1 (0.5%)	11/18 (61%)
Step 2 (3%)	16/18 (89%)
Controls	No reactions were seen
Other information	Difference between test and control group statistically significant

Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) (8)	
Design	blinded, randomised and controlled
Test subjects	15 patients with a positive patch test to HICC
Controls	10 healthy controls
Substance	Purity: > 98.8%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	6% to 0.0006% (5 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions
ROAT	Axilla
area	76 cm ² (template)
applications/day	two with roll on deodorant
dose	Step 1: 200 ppm Step 2: 600 ppm Step 3: 1800 ppm
dose/application/cm ²	median 0.79 µg HICC
control substance	deodorant matrix
definition of positive	spotty erythema involving at least 25% of the exposed area and infiltration represented by at least one papule.
period	two weeks for each step; total maximum 6 weeks
Results	
PT ED10% (95% CI)	0.75 µg/cm ² 25 ppm (0.69-120)
PT ED50% (95% CI)	18.3 µg/cm ² 610 ppm (120-2800)
PT no effect level (observed)	< 0.0006%
ROAT	Cumulative responses
Step 1 (200 ppm)	9/14* (64%)
Step 2 (600 ppm)	12/14* (86%)
Step 3 (1800 ppm)	14/14* (100%)
Controls	No reactions were seen
Other information	*14 patients completed the use test study Difference between HICC deodorant and matrix deodorant in cases ($p=0.0001$). Difference between controls and patients ($p=0.004$).

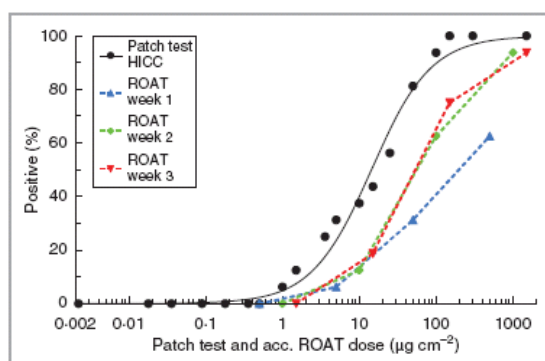
Hydroxyisohexyl 3-cyclohexenecarboxaldehyde (HICC) (9)	
Design	blinded, randomised and controlled
Test subjects	17 patients with a positive patch test to HICC
Controls	15 healthy controls
Substance	IFF lot SM/8059062
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	1500 to 0.0022 µg/cm ² HICC (19 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions to higher concentrations
ROAT	volar aspect of forearms
area	3 x 3 cm (5 areas)
applications/day	two with micropipette (20 µl per application)
dose	Simultaneous application to 5 areas, four doses each and vehicle
µg /application/cm ²	Dose 1:0.0357 Dose 2: 0.357 Dose 3: 3.57 Dose 4: 35.7
control substance	ethanol
definition of positive	at least 5 points on a clinical scale, corresponding to erythema in 25% of test area and at least 1 papule
period	Three weeks. All concentrations applied simultaneously (randomised)
Results	
PT ED10% (95% CI)	0.662 µg/ cm ² (0.052-2.35)
PT ED50% (95% CI)	11.1 µg/ cm ² (3.41- 33.1)
PT no effect level(observed)	<0.0022 µg/ cm ²
ROAT	Cumulative responses
Dose 1 (0.0357)	0/16*
Dose 2 (0.357)	3/16 (19%)
Dose 3 (3.57)	12/16 (75%)
Dose 4 (35.7)	15/16 (94%)
Controls	No reactions were seen
Other information	*16 patients completed the use test study The evaporation rate of HICC was calculated to 72% over a 24-h period. ED10% ROAT: 0.064 µg/cm ² (more info see below)

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Table 2 The dose per application and accumulated dose after 1, 2 and 3 weeks in the ROAT

ROAT, dose per application ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 1 week	Total accumulated dose after 1 week ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 2 weeks	Total accumulated dose after 2 weeks ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 3 weeks	Total accumulated dose after 3 weeks ($\mu\text{g HICC cm}^{-2}$)
35.7	14	500	28	1000	42	1500
3.57	14	50	28	100	42	150
0.357	14	5	28	10	42	15
0.0357	14	0.5	28	1	42	1.5

ROAT, repeated open application test; HICC, hydroxyisohexyl-3-cyclohexene carboxaldehyde.

Fig 3. The fitted dose-response curve for the patch test ($n = 16$) and the 1-week, the 2-week 3-week accumulated ROAT doses.

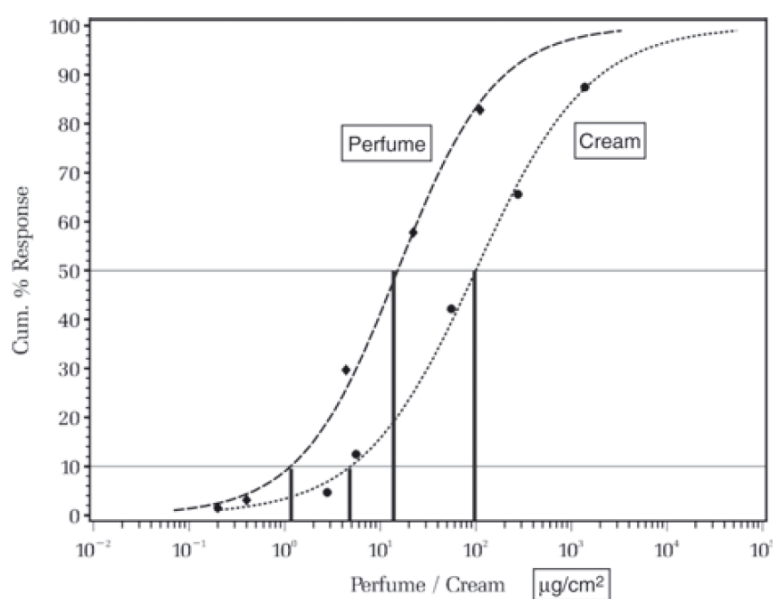
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

In a study by the German Contact Dermatitis Group, 64 persons previously diagnosed with HICC contact allergy were exposed to increasing doses of HICC in 2 different formulations, a hydrophilic cream and an ethanol solution, to mimic everyday exposures, following a standardised ROAT protocol (10). The concentration of HICC tolerated by 90% of the sensitised was estimated as 1.2 µg/cm² for perfume and 4.9 µg/cm² for cream. The dose-response curve is shown in Fig. 4.3 – 1 below.

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (10)			
Design	randomised and vehicle controlled		
Test subjects	67 patients with a previous positive patch test to HICC		
Controls	None		
Substance	Provided by International Flavor & Fragrances Inc, Hilversum, NL		
Patch test			
-dilution steps	2.5% and 5%		
-control/vehicle	petrolatum		
-definition of threshold	lowest concentration giving a positive skin reaction in a continuous line to next higher concentration.		
ROAT	Volar forearms (both sides)		
area	3 x 3 cm (4 areas: one test and one control each for alcoholic solution and cream, respectively)		
applications/day	two		
dose	2.8 µg/cm ² in cream	0.2 µg/cm ² in ethanol	
	5.6 µg/cm ² in cream	0.4 µg/cm ² in ethanol	
	55.6 µg/cm ² in cream	4.4 µg/cm ² in ethanol	
	277.8 µg/cm ² in cream	22.2 µg/cm ² in ethanol	
	1388.9 µg/cm ² in cream	111.1 µg/cm ² in ethanol	
µg /application/cm ²	See above		
control substance	Ethanol 96% and glyceryl stearate 15% in water, resp.		
definition of positive	(spotty) erythema of at least 25% of the test area along with homogeneous infiltration or papules regardless of the number		
period	Two weeks for each step until positive reaction or end of study, whichever occurred first		
Results			
PT ED10% (95% CI)	Not calculable; 52 of 60 Patients patch tested positive to 2.5% HICC, 57 / 60 to 5% HICC		
PT ED50% (95% CI)	Not calculable		
PT no effect level (observed)	Not calculable		
ROAT	Cumulative responses:		
	Cream preparation: 2.8 µg/cm ² : 4.7%	Ethanol preparation: 0.2 µg/cm ² : 1.6%	

	5.6 $\mu\text{g}/\text{cm}^2$: 12.5% 55.6 $\mu\text{g}/\text{cm}^2$: 42.2% 277.8 $\mu\text{g}/\text{cm}^2$: 65.6% 1388.9 $\mu\text{g}/\text{cm}^2$: 87.5%	0.4 $\mu\text{g}/\text{cm}^2$: 3.1% 4.4 $\mu\text{g}/\text{cm}^2$: 29.7% 22.2 $\mu\text{g}/\text{cm}^2$: 57.8% 111.1 $\mu\text{g}/\text{cm}^2$: 82.8%
Controls	No reactions to vehicle in the patients included into analysis	
Other information	See figure below. Three patients were excluded from the study, so results are based on 64 patients.	

Figure 4.3 – 1: Dose-response curve of 64 patients sensitised to HICC, according to a previous PT, regarding two preparations: perfume and cream, the rhomboid and dot symbol, respectively, indicating the observed response. The curve was fitted by a logistic function (10).



Isoeugenol

Isoeugenol (11)	
Design	blinded, randomised doses and controlled
Test subjects	20 patients with a positive patch test to isoeugenol
Controls	20 healthy controls
Substance	purity: 98%
Patch test	20 mg solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.01% (8 steps)
-control/vehicle	petrolatum
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line
ROAT	outer aspect of upper arms
area	5 x 5 cm (2 areas: one test and one control)
applications/day	two with roll-on
dose	0.2% in ethanol
μg /application/ cm^2	Doses measured to 0.14 -0.13 mg/application the first 14 days = 5.6 $\mu\text{g}/\text{cm}^2$
control substance	ethanol
definition of positive	any degree of reaction
period	Two weeks at upper arm and if negative another two weeks including application to base of neck
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	0.08% 32 $\mu\text{g}/\text{cm}^2$
PT no effect level (observed)	< 0.01% = 0.4 $\mu\text{g}/\text{cm}^2$
ROAT	
Dose: 0.2%	12/19 (63%)
Controls	No reactions were seen
Other information	

Isoeugenol (12)	
Design	blinded, randomised
Test subjects	27 patients with a positive patch test to isoeugenol
Controls	20 healthy controls
Substance	purity: 98%
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.00006% (17 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration giving a visible skin reaction in a continuous line of reactions to higher concentrations
ROAT	volar aspect of lower arm
area	3 x 3 cm (2 areas)
applications/day	two with droplet bottle (30 mg per application)
dose	0.05% in ethanol and 0.2%
µg /application/cm ²	Doses were calculated as mean 2.2 µg/cm ² (low conc.) and 9 µg/cm ² (high conc.)
control substance	ethanol
definition of positive	clear visible erythema
period	28 days
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level (observed)	< 0.0005% (5 ppm)
ROAT	Cumulative responses
Dose 1: 0.05%	10/24 (42%)
Dose 2: 0.2%	16/24 (67%)
Controls	No reactions were seen
Other information	Response to the low concentration in the ROAT appeared after median 15 days and to the high concentration after median 7 days.

Isoeugenol (13)	
Design	blinded, randomised and controlled
Test subjects	13 patients with a positive patch test to isoeugenol and 4 in part 1 (pre-test)
Controls	10 healthy controls (dermatitis patients)
Substance	purity: /
Patch test	15 µl solution applied in an 8 mm Finn Chamber occlusion 48 h
-dilution steps	2% to 0.00006% (w/v) (16 steps)
-control/vehicle	ethanol
-definition of threshold	lowest concentration eliciting at least + reaction
ROAT	Axilla
area	10 x 10 cm ² (estimated)
applications/day	two with roll-on deodorant (117-586 mg per application of solution) average cases: 266 mg/application controls: only range given
dose	Part 1: Step 1:0.02% Step 2: 0.063% Step 3:0.2% Part 2: Step1:0.0063% Step 2:0.02% Step 3: 0.063%
dose/application/cm ²	Part 2: Step 1: 0.167 Step 2: 0.53 Step 3: 1.67 µg/application/cm ² (calculated based on data)
control substance	deodorant matrix
definition of positive	eczematous response covering 25% of test area
period	Part one: one week with each concentration: maximum three weeks Part two: two weeks with each concentration: maximum six weeks
Results	
PT ED10% (95% CI)	/
PT ED50% (95% CI)	/
PT no effect level (observed)	<0.0005% (0.15 µg/cm ²)
ROAT	
Step 1 (0.0063%)	3/13 (23%)
Step 2 (0.02%)	9/13 (69%)
Step 3 (0.063%)	10/13 (77%)
Controls	No reactions were seen
Other information	Deodorants containing cinnamal were responsible for all reactions in cinnamal sensitized individuals ($p<0.001$) and all control persons were negative ($p<0.001$)

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Tsai, Victoria

From: Katz, Linda
Sent: Thursday, June 25, 2015 10:47 AM
To: Ikhlas Khan (ikhlan@olemiss.edu)
Cc: Hansen, Patricia A; Sadrieh, Nakissa; Milstein, Stanley R
Subject: FW: list
Attachments: 26 EU Fragrance Allergens. 6-17-15.ppt; 26 Fragrance Allergens. ICCR WG-Allergens. f.pdf; FRAGRANCE ALLERGENS IN COSMETIC PRODUCTS. sccs_o_102 .6-26-12.pdf

Ikhlas,

Attached is the list of the 26 EU fragrance allergens as well as the SCCS opinion. Some of these substances are either confirmed contact allergens or presumptive contact allergens (but not confirmed); other tables present those which are suspected to be human contact allergens or which are based on animal, in-vitro (LLNA), or QSAR/*in-silico* data. Table 13-5 presents 12 established chemical fragrance allergens with high risk of sensitization to humans. In the SCCS Opinion, the Threshold exposure (for single chemicals only, not extracts or mixtures) which could be tolerated by most consumers is estimated to be $\leq 0.8 \mu\text{g}/\text{cm}^2$.

Chemical of special concern include: tree moss, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), oak moss, isoeugenol, hydroxycitronellal, citral, cinnamal, farnesol and cinnamyl alcohol. However, we are interested in the remainder of those listed below from Table 13-5.

Cinnamal
Cinnamyl Alcohol*
Citral
Coumarin
Eugenol*
Farnesol*
Geraniol*
Hydroxycitronellal
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)
Isoeugenol*
Limonene (oxidised)
Linalool* (oxidised)
*including their respective esters

The 2012 SCCS Opinion also recommends that fragrance pre-haptens and pro-haptens of several terpene fragrance materials should be considered as putative "allergens" and regulated in the same way as the allergens by the EC (Among them are limonene, linalool, linalyl acetate, geraniol, geranial, α -terpinene, eugenol, isoeugenol, and cinnamyl alcohol).

Let me know if you need any further information at this time regarding allergens.

On a different note, let me know if you have a report and list with description of in vitro assays, including validation, that actually describes the methods that you have developed for arbutin.

Linda

Tsai, Victoria

From: Katz, Linda
Sent: Wednesday, July 25, 2018 2:34 PM
To: Tsai, Victoria
Subject: FW: Rose Sheet article re: CIR priorities

I just came across this one. The others I will try and bundle and put into the F: drive.

From: Sadrieh, Nakissa
Sent: Thursday, June 14, 2018 4:15 PM
To: Katz, Linda <Linda.Katz@fda.hhs.gov>
Subject: FW: Rose Sheet article re: CIR priorities

Please see the mail below from Ikhlas. Should I (b) (5) ?

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
5001 Campus Drive
Room 1042 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Thursday, June 14, 2018 3:03 PM
To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Subject: FW: Rose Sheet article re: CIR priorities

Hi Nakissa

I am sure you know them. Should we participate and assist or just keep doing science and be safe. What do you think?
IK

Tsai, Victoria

From: Katz, Linda
Sent: Thursday, August 16, 2018 12:12 PM
To: Tsai, Victoria
Subject: FW: Thanks

Not sure if I sent this before. Do you still need me to look for more?

From: Ikhlas Khan <ikhan@olemiss.edu>
Sent: Wednesday, May 23, 2018 3:41 PM
To: Katz, Linda <Linda.Katz@fda.hhs.gov>
Subject: Re: Thanks

Funding always fluctuate but your support is more important. Appreciate it. Hope we can have more in future

Thanks a lot and please feel free to contact us if we can be any assistance
Ik

Sent from my iPhone

On May 23, 2018, at 1:30 PM, Katz, Linda <Linda.Katz@fda.hhs.gov> wrote:

Unfortunately we don't have any extra money this year and only allotted for \$250,000.

Linda

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Wednesday, May 23, 2018 2:09 PM
To: Katz, Linda <Linda.Katz@fda.hhs.gov>
Subject: Re: Thanks

Dear Linda

Thanks for quick response, yes conference went very well. You should attend next year if your time permits.
The dates are 8-11, April 2019.

Thanks for your continued support, I believe last year you initially allocated 250K but later on 100k was added, total \$350k. I know you will do best to keep us running.
Appreciate it.
ik

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Wednesday, May 23, 2018 at 12:25 PM
To: Ikhlas Khan <ikhan@olemiss.edu>
Subject: RE: Thanks

Ikhlas,

I'm doing well and hoping you are as well. I heard from Stan that the meeting in April was a success. We appreciate all of the work that you have done with us this past year. We have again contributed \$250,000 towards our continued collaboration.

If you need anything else, let me know.

Linda

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Wednesday, May 23, 2018 12:34 PM
To: Katz, Linda <Linda.Katz@fda.hhs.gov>
Cc: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Subject: Re: Thanks

Dear Linda

Hope you are doing well. I am sure you are being informed about the progress we are making and Nakissa is helping all of us to work together efficiently. We are making good progress. This is the time of the year again to ask for your continuous support for the collaboration. I hope you can support us at the same level as last year.

Thanks again

Ik

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Thursday, July 27, 2017 at 11:51 AM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Subject: RE: Thanks

Glad things worked out for this year.

Linda

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Thursday, July 27, 2017 12:19 PM
To: Katz, Linda
Cc: Sadrieh, Nakissa
Subject: Thanks

Dear Linda

I just heard from Cara, I do appreciate your additional support.

Thanks

IK

Application of UM's *in chemico* methods for Estimating the Sensitization Potential

Case studies on Chamomile and Tea Tree Oil



Case Study#1

Skin sensitization potential of chamomile



NOPSIS

Two most popular and commonly used chamomiles

German chamomile (*Matricaria chamomilla*, synonym: *Matricaria recutita*)

Roman chamomile (*Chamaemelum nobile*, synonym: *Anthemis nobilis*)



Matricaria chamomilla



Chamaemelum nobile

➤ **Aim:**

1. Which chamomile? Roman, German or Chinese?
2. Skin sensitization potential?

➤ **Methodologies applied:**

GC-MS

UPLC-QToF

HPTLC

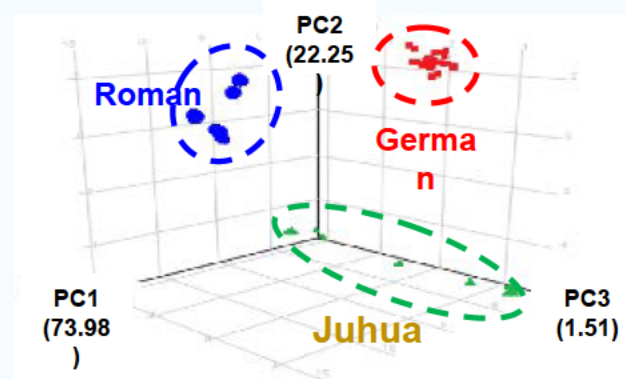
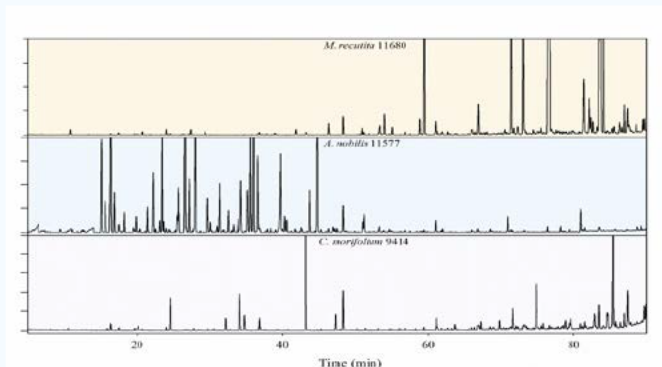
HTS-DCYA

NMR-DCYA

KeratinoSens™

Classification of Chamomiles Using GC/MS and Chemometrics

➤ Results:



- A highly accurate statistical model has been developed to determine the exact type (German, Roman and Juhua) of chamomile used in commercial herbal products and dietary supplements.
- The model was developed from GC/MS data. Quality control of the samples was performed by Principal Component Analysis (PCA).
- A sample class prediction model based on Partial Least Squares Discriminant Analysis (PLS-DA) was constructed.
- The results suggested that German chamomile is the major type of chamomile used in the U.S. market.

Classification of Chamomiles Using UHPLC-UV-MS

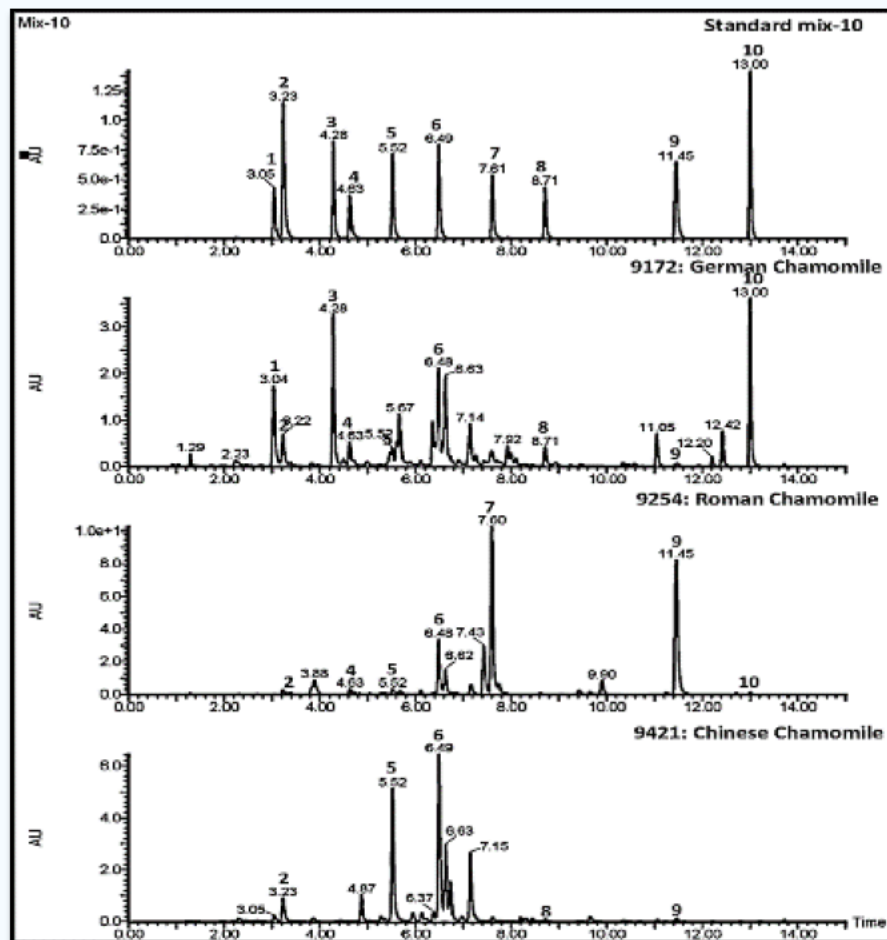
Results:

Standard Mix-10

German chamomile

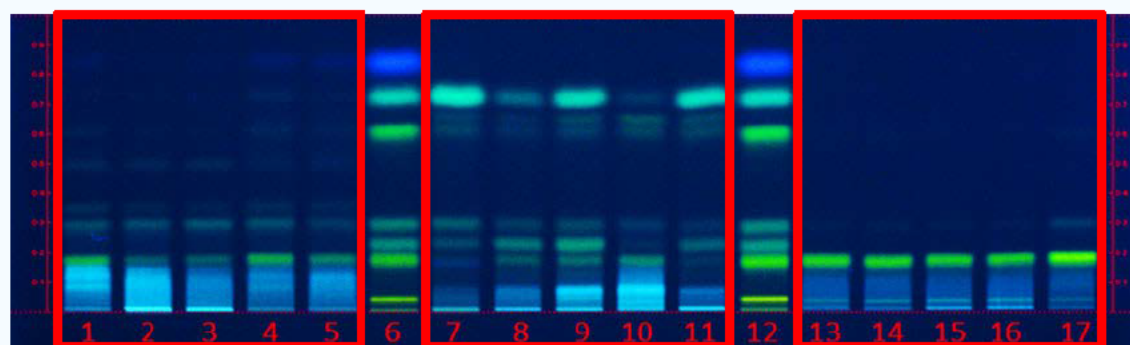
Roman chamomile

Chrysanthemum morifolium



Classification of Chamomiles Using HPTLC

➤ Results:

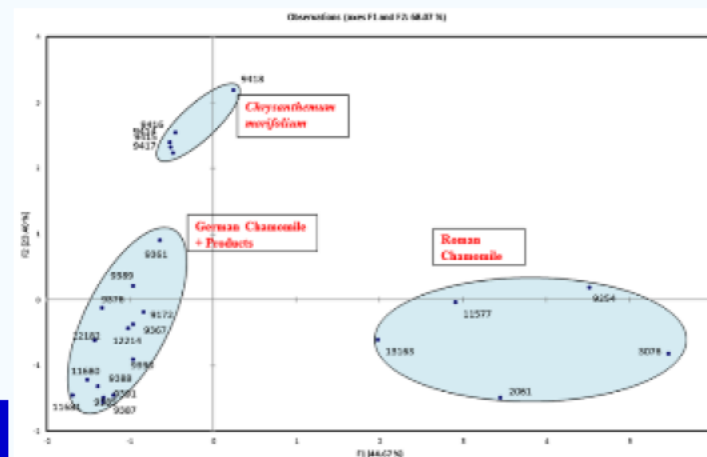


German
chamomile

Roman
chamomile

Juhua

Reference standards (Rf order): rutin (1), luteolin-7-O-glucoside (2), chamaemeloside (3), apigenin-7-O-glucoside (4), luteolin (5), apigenin (6), and umbelliferone (7)



PCA score plots for authenticated chamomile samples and *Chrysanthemum*

Determination of Sensitization Agents Within Chamomile Species

➤ Results: KeratinoSens™ data

EC1.5 and IC₅₀ Summary Table

Assay Date	HIVS Test Article	Sponsor Designation	EC 1.5 value [^] (μg/mL)	Mean IC ₅₀ (μg/mL)		Potential Sensitizer? [†]
				MTT	NRU	
	11AH38	German Chamomile Hexane Fraction (9172 Hexane, JZ-11A-2-2)	0.67	168	160	YES
	11AH39	German Chamomile Chloroform Fraction (9172 CHC13, JZ-11A-13-2)	5.85	155	72.6	YES
	11AH40	German Chamomile Ethanol extract (3760 EtOH, IKX-1-55.11)	21.23	> 400	> 400	YES
26 July 2011	11AH41	Roman Chamomile Hexane Fraction (9254 Hexane, JZ-11A-10-2)	2.66	88.5	81.4	YES
	11AH42	Roman Chamomile Chloroform Fraction (9254 Hexane, JZ-11A-10-3)	0.50	9.96	8.10	YES
	11AH43	Chamomile Essential oil (9369)	2.23	9.47	9.44	YES
	11AH44	Bisabolol	> 400	9.38	9.30	NO
7 Sept 2011	Chloroform	Chloroform	> 400	> 400	> 400	NO
	Hexane	Hexane	> 400	> 400	> 400	NO
26 July 2011	Cinnamic Aldehyde	Positive Control	10.26 μM	> 64 μM	> 64 μM	YES
7 Sept 2011			8.16 μM	> 64 μM	> 64 μM	YES

EC1.5 and IC₅₀ Summary Table

Assay Date	HIVS Test Article Number	Sponsor's Designation	EC 1.5 value [^] (μg/mL)	Mean IC ₅₀ (μg/mL)		Potential Sensitizer? [†]
				MTT	NRU	
	12AE99	JZ-12-11-3, German Chamomile Extract	3.29	>400	377	YES
	12AF00	JZ-12-15-1, German Chamomile Fraction	2.96	291	352	YES
	12AF01	JZ-12-14-3, German Chamomile Fraction	3.02	336	>400	YES
15 May 2012	12AF02	JZ-12-14-4, German Chamomile Fraction	0.471	>400	>400	YES
	12AF03	JZ-12-14-5, German Chamomile Fraction	24.8	279	290	YES
	12AF04	JZ-12-14-6, German Chamomile Fraction	34.8	>400	>400	YES
	12AF05	JZ-12-7-3, Roman Chamomile Extract	0.718	83.5	80.7	YES
	Cinnamic Aldehyde	Positive Control	13.3	>64	>64	YES

Determination of Sensitization Agents Within Chamomile Species

➤ Results:

- German and Roman Chamomile extracts along with fractions were evaluated for sensitization potential using KeratinoSens™ assay by a commercial laboratory (IIVS).
- Several fractions are found to have sensitization potential without any cytotoxicity.
- Direct Peptide Reactivity Assay failed to estimate the sensitization potential of the pure compounds of German Chamomile due to their solubility in only DMSO. The solvent, DMSO was found to have detrimental effects on Cys-DPRA results.

Determination of Sensitization Agents Within Chamomile Species

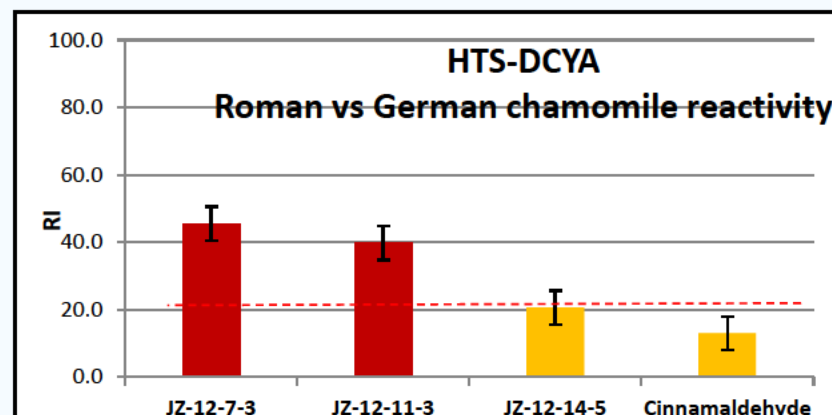
➤ Results:

- Authenticated German (*Matricaria recutita*) and Roman chamomile (*Chamaemelum nobile*) samples were screened with DCYA-HTS method and compared with KeratinoSens™ data.
- Roman chamomile (RC) crude extract resulted in a stronger response compared to German chamomile (GC) extract and enriched fractions
- The results obtained with the DCYA-HTS method were comparable to in vitro results

Sample	Description	HTS	Keratinosens ¹
		RI*	IC ₅₀
JZ-12-7-3	Roman Chamomile Extract	45.5	0.718
JZ-12-11-3		39.8	3.29
JZ-12-14-5	German Chamomile Fraction	20.6	24.8
Cinnamaldehyde	Positive control	14.9	13.3

*Note: for HTS results, the higher the RI, the stronger the sensitizer

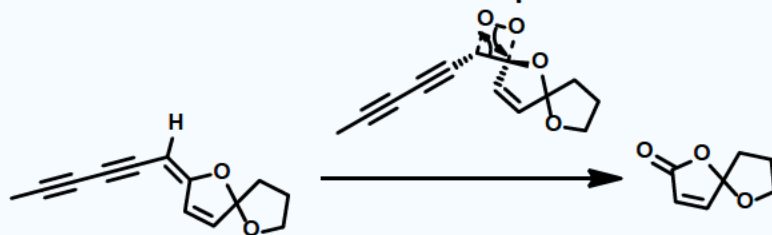
¹See Report Jul 31st 2013- study number 12AE99-AF05.170000, Institute for In Vitro Sciences,



Determination of Sensitization Agents Within Chamomile Species

➤ Results:

- **Tonghaosu** is one of the main components of **German** chamomile
- The compound possesses several structural alerts and can be considered as a potential hazard as it has **structure resemblance to known sensitizers** (e.g. falcarinol)¹
- Tonghaosu was **non-reactive** under DCYA-NMR and DCYA-HTS methods
- Identified unstable nature of tonghaosu, isolated oxidative degradation product and characterized as dioxo-spiro lactone.

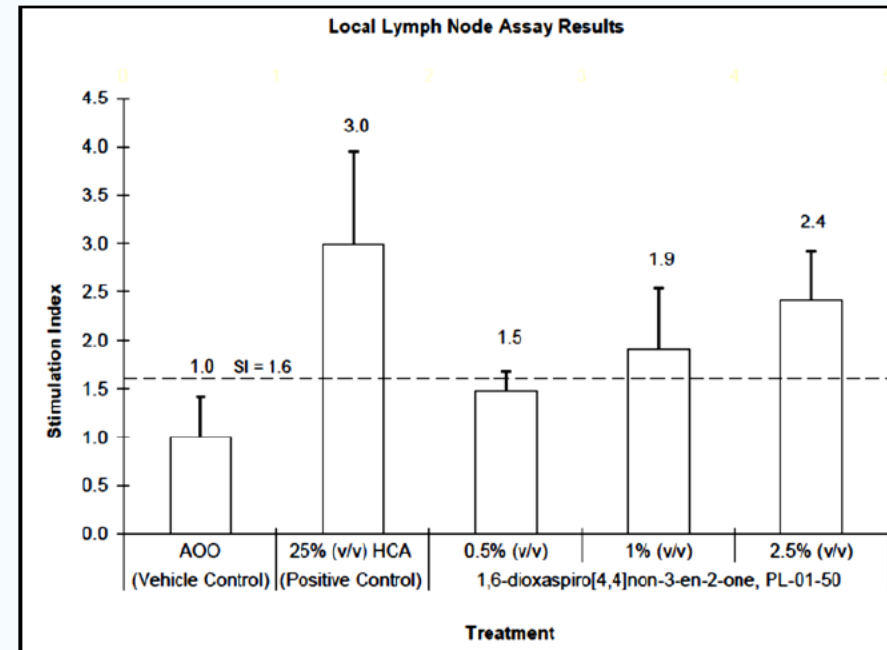


Determination of Sensitization Agents Within Chamomile Species

➤ Results:

LLNA data

- In order to confirm the sensitization potential of the identified lactone, several grams of the compound was synthesized to meet the sample requirements for LLNA. In vivo studies were performed by an external laboratory¹
- The compound was tested at different concentrations, and classified as a dermal sensitizer



¹MB Research Laboratories, Research Project MB # 14-23242.26

Determination of Sensitization Agents Within Chamomile Species

➤ Outcomes :

- According to WHO monogram on German chamomile, very few cases of allergy were specifically attributed to German chamomile.¹ Adverse effects were attributed to presence of Lactones
- Tonghaosu is one of the major marker compound in essential oil of German chamomile
- Several commercial EOs of German chamomiles were studied and 50-160 mg of Tonghaosu per gram of EO was observed.
- Tonghaosu undergoes oxidative transformation into potential sensitizer (dioxo-spiro lactone) and can be considered a pre-hapten. The sensitization potential was confirmed with LLNA.

¹Hausen BM, Busker E, Carle R. Über das Sensibilisierungsvermögen von Compositenarten. VII. Experimentelle Untersuchungen mit Auszügen und Inhaltsstoffen von *Chamomilla recutita* (L.) Rauschert und *Anthemis cotula* L. *Planta medica*, 1984:229–234.

Case Study #2

Authentication and safety concern of Tea Tree Oil



SYNOPSIS

- Essential oil obtained by steam distillation of the foliage and terminal branch lets of *Melaleuca alternifolia*, *Melaleuca linariifolia*, *Melaleuca dissitiflora* or other species of the genus *Melaleuca*
- Due to the increasing market for Tea Tree Oil (TTO), there is a growing trend toward adulteration or substitution of these products
- A total of 104 samples were provided by ATTIA Ltd (Australia) and analyzed for authenticity
- *In vivo* studies (LLNA) confirmed a stronger sensitization potential for aged TTOs compared to fresh ones. A number of constituents of TTO have been suggested as potential candidate sensitizers due to chemical or metabolic transformation in *in vivo*
- Ten different TTO and related species have been investigated for the sensitization potential after aging along with major components of TTO

➤ **Aim:**

1. Development of analytical methods to distinguish authentic TTO from other tea tree species
2. Investigation of the sensitization potential of TTO and related species using HTS-DCYA assay with crude EO mixtures
3. Investigation of the sensitization potential upon aging of individual TTO main constituents

➤ **Methodologies applied:**

- GC-MS coupled to PCA
- HTS-DCYA

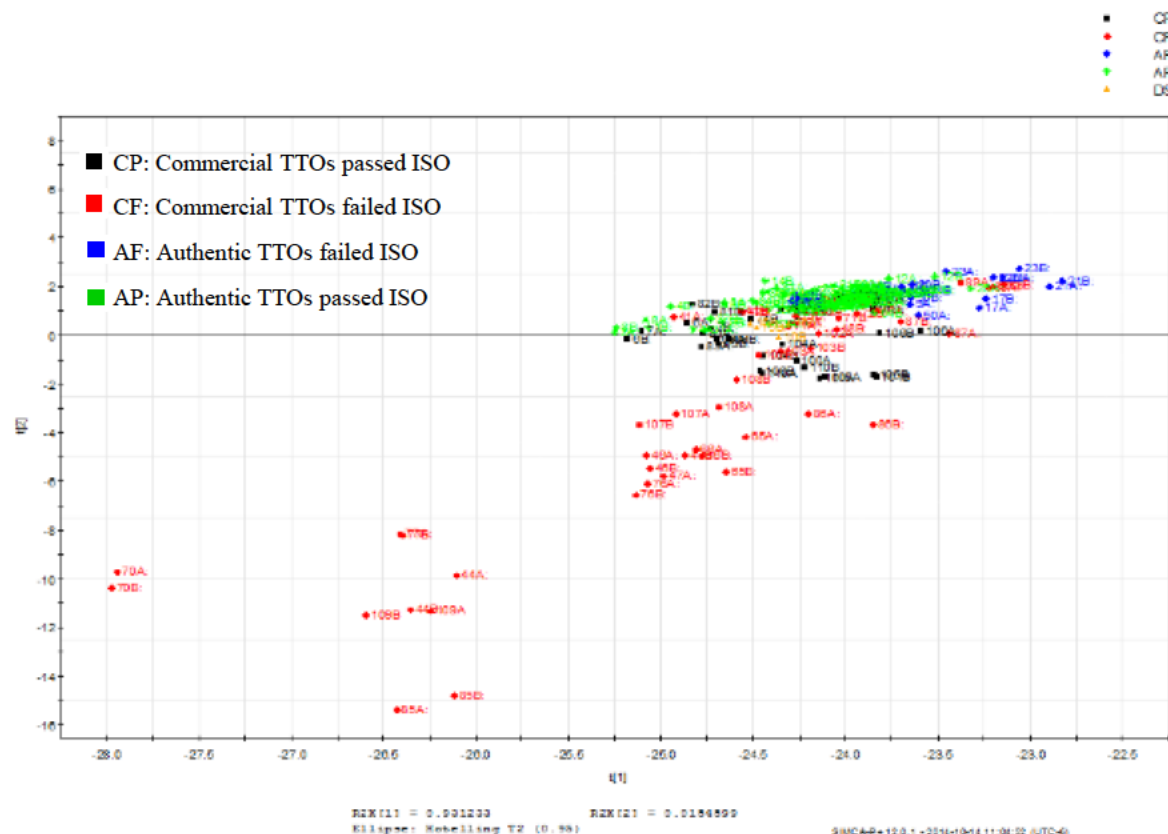
Identification of the Major Compounds in Tea Tree Oil

Peak No.	Compound	t _R	%
1	α -thujene	16.10	1.01
2	(-)- α -pinene	18.67	0.27
3	(+)- α -pinene	19.05	3.02
4	β -myrcene	19.85	0.44
5	β -pinene	21.97	0.52
6	α -terpinene	22.12	5.83
7	p-cymene, (\pm) limonene	23.26	25.07
8	(\pm)- β -phellandrene	24.01	0.21
9	(\pm)- β -phellandrene	24.19	0.37
10	γ -terpinene	24.78	17.27
11	α -terpinolene	25.73	5.61
12	1,8-cineole	26.07	2.88
13	(+)-terpinen-4-ol	34.64	16.35
14	(-)-terpinen-4-ol	34.53	9.36
15	(-)- α -terpineol	36.41	0.36
16	(+)- α -terpineol	36.56	1.25
17	α -copaene	37.07	0.20
18	p-cymene-8-ol	37.93	0.29
19	α -gurjunene	39.03	0.20
20	β -caryophyllene	40.65	0.25
21	alloaromadendrene	41.66	0.85
22	aromadendrene	42.61	0.32
23	viridiflorene	44.50	0.69
24	δ -cadinene	46.02	1.19
25	globulol	51.92	0.51
26	viridiflorol	52.45	0.58
Total			94.87



PCA analysis for authentication of TTO

- All the authentic TTOs that met the ISO standards cluster in a single group (green) in the PCA score plot.
- The chiral ratios of α -pinene, limonene, terpinen-4-ol and α -terpineol for the tea tree oil samples in this group were also within the ranges of norms.



Identification of the Major Compounds in Tea Tree Oil

➤ Outcome :

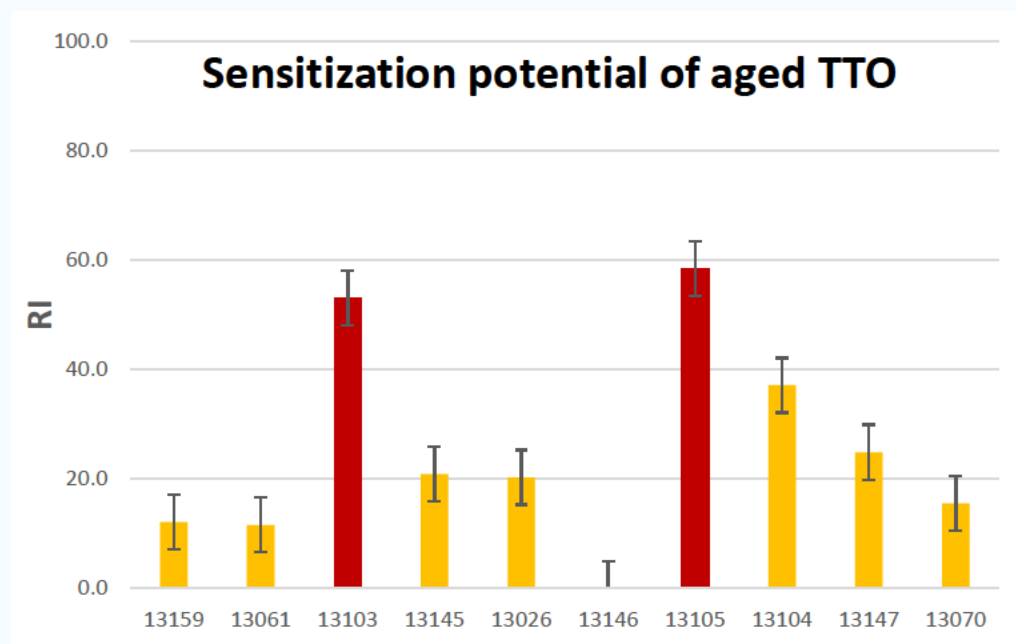
- A GC/MS method was developed for the analysis of the enantiomeric compounds in tea tree oil.
- A total of 104 samples from ATTIA Ltd., Australia were acquired. The ratios of four enantiomeric compounds were determined.
- The distribution of enantiomeric ratios were utilized for establishment of quality and authenticity of the tea tree oil.
- Based on GC-MS data, Principle Component Analysis was conducted and a model was developed for establishing the authenticity of TTO. The use of a chemometric tool, such as PCA, can produce complementary information to chiral GC.

Sensitization Potential of TTO

- Ten TTO samples of *M. alternifolia* and related species previously analyzed by our group were undertaken for the aging study
- Aged samples were then characterized by GC/MS and compared data to the original chemical composition
- The content of *p*-cymene and hydrogen peroxide were quantified as indication of aging
- The aged oils were then assessed for their sensitization potential using the DCYA-HTS method.

Sensitization Potential of TTO

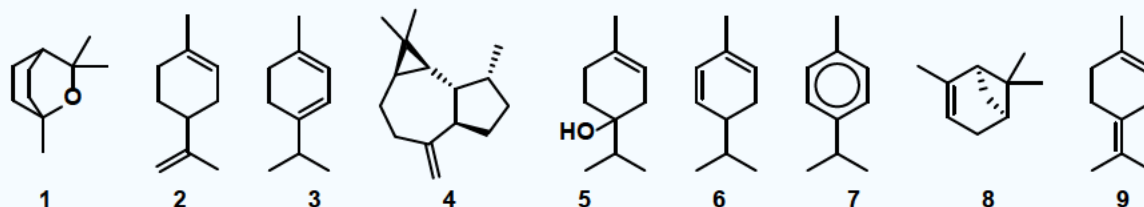
- From the DCYA-HTS results, some oils resulted stronger reactive indices indicating the higher sensitization potential
- With regard to authentic TTO (*M. alternifolia* and *M. linariifolia*), a correlation between content of *p*-cymene and sensitization potential was found



	PLANT SOURCE
# 13159	Melaleuca alternifolia
# 13061	Melaleuca cajuputi
# 13103	Melaleuca leucadendron
# 13145	Melaleuca
# 13026	Melaleuca linariifolia
# 13146	Leptospermum scoparium
# 13105	Melaleuca alternifolia
# 13104	Melaleuca viridiflora
# 13147	Kunzea ericoides
# 13070	Leptospermum + Melaleuca

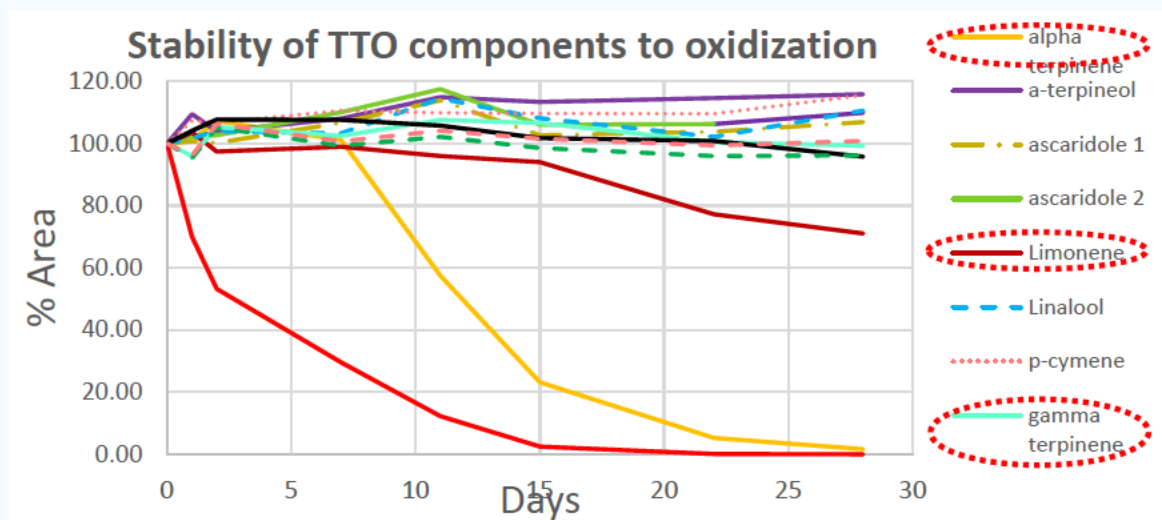
Sensitization Potential of TTO's Components

- As a clear correlation between the monoterpenes content, aging and potential sensitization was found, the principal TTO components (1-9) were also investigated for their sensitization potential

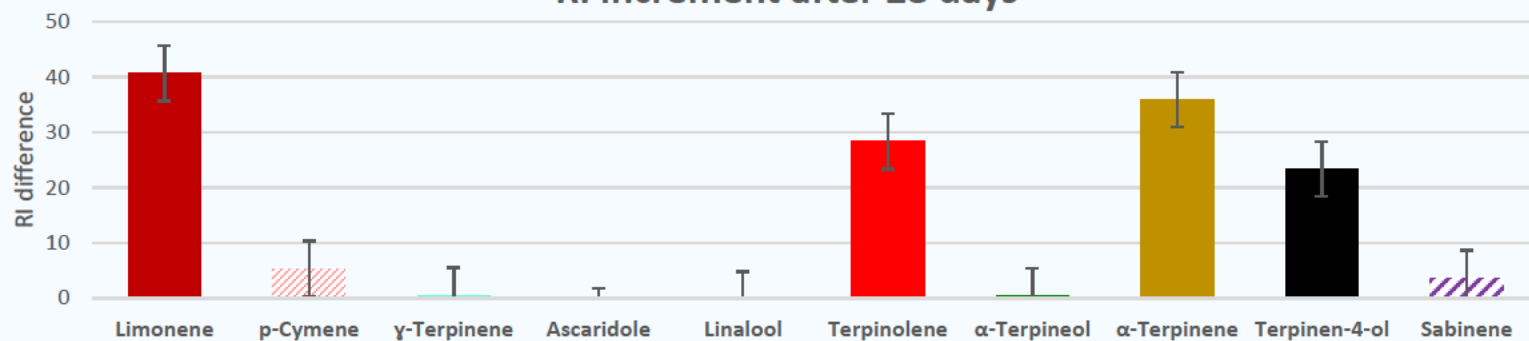


- The pure compounds were subjected to expedited aging by continuous bubbling of oxygen for 28 days.
- Several aliquots were collected (0-28 days), analyzed for their stability with GC/MS and subjected to UM-HTS method for their sensitization potential
- Terpinolene**, **α -terpinene** and **limonene** were found to be most unstable under these conditions

Sensitization Potential of TTO's Components



RI increment after 28 days



Sensitization Potential of TTO and its Major Components

➤ Outcomes:

- GC-MS method and PCA model were developed for to establish the authenticity of Tea Tree Oils
- A strong correlation between aging and sensitization potential of genuine TTOs was observed
- Non-aged pure TTO components were found non- to weakly reactive
- Upon accelerated aged conditions, increased sensitization potential was observed and the chemical reactivity is proportional to the degradation ability of each component.
- The obtained results are in agreement with the literature reports on skin sensitization potential of oxidized TTO and its components.
- The DCYA-HTS method served as a useful tool in estimating the sensitization potential of complex essential oils, viz., Tea Tree Oils

Annex II
Comparative LLNA, Guinea Pig, and Human Data
Used in the Performance Evaluation

Annex II-1	
LLNA Data for 196 Substances Used for the Evaluation of Skin Sensitization Potency (Alphabetical Order)	C-123
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Human Data for LLNA Potency Evaluation	C-211
Annex II-3	
Guinea Pig Data for LLNA Potency Evaluation	C-233
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Summary LLNA, Human, and Guinea Pig Data Used in the Regression and Classification Rate Analyses	C-253

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Annex II-1

**LLNA Data for 196 Substances Used for the Evaluation of Skin Sensitization Potency
(Alphabetical Order)**

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Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Abietic acid	AOO	5.0 10.0 25.0	1.5 2 5.2	15.0	3750	N	CBA	NA	(Ashby et al. 1995)
Abietic acid	AOO	NA	NA	11.0	2750	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003b)
Abietic acid	AOO	NA	NA	14.7	3675	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2007)
Abietic acid	AOO	NA	NA	8.3	2075	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2007)
Abietic acid	AOO	NA	NA	10.6	2650	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2007)
Acetyl isovaleryl ¹	AOO	25 50 100	2.9 6 14.3	25.8	6450	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2000)
AE F016382 00 TK71 A101	Pluronic L92	3.6 7.1 17.9 35.7	1.0 0.8 1.0 1.1	NC	NC	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
Aluminum chloride	Pet.	5 10 25	0.8 0.8 0.7	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999b)
p-Aminobenzoic acid	AOO	0.5 1 2.5 5 10	1.2 1.2 1.1 1.6 1.4	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)

¹ The reference refers to this substance as 5-methyl-2,3-hexanedione.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
3-Aminophenol	AOO	2.5 5.0 10.0	2.8 3.5 5.7	3.2	800	Y ²	CBA/Ca	NA	(Basketter and Scholes 1992)
3-Aminophenol	DMF	NA	NA	0.2	60	N	NA	NA	(SCCP 2007)
Amylcinnamic aldehyde	NA	NA	NA	12.1	3013	NA	NA	NA	(Estrada et al. 2003)
Amylcinnamic aldehyde	AOO	NA	NA	13.5	3375	N	CBA	NA	(Basketter and Cadby 2004)
Amylcinnamic aldehyde	AOO	1.0 2.5 5.0 10.0 25.0	1.5 1.7 2.2 2.8 8.2	10.6	2650	N	NA	NA	(Patlewicz et al. 2001)[EC3] (Gerberick et al. 2005)[Dose-response data]
Amylcinnamic aldehyde	EtOH/DEP (1:3)	NA	NA	7.6	1900	N	NA	NA	(RIFM 2007)
Amylcinnamic aldehyde	AOO	NA	NA	11.2	2800	NA	NA	NA	(Smith and Hotchkiss 2001)
alpha-Amylcinnamyl alcohol	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
alpha-Amylcinnamyl alcohol	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Aniline	AOO	NA	NA	37.0	9250	NA			(Griem et al. 2003) (Smith and Hotchkiss 2001)
Aniline	AOO	5.0 10.0 25.0 50.0 100.0	1.1 0.9 2.0 1.9 3.3	89	22250	N	CBA	NA	(Gerberick et al. 2005)

² Protocol used both sexes, and the test duration was 4 or 5 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Aniline	AOO	10 25 50	1.4 1.8 2.9	NC	NC	Y ³	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Basketter et al. 1991) (Basketter and Scholes 1992)
Aniline	MEKOO	10 25 50	1.2 1.5 1.7	NC	NC	Y ⁴	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Basketter et al. 1991)
Aniline	MEK	10 25 50	1.5 1.7 3	50.0	12500	Y ⁵	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Basketter et al. 1991)
Aniline	AOO	10 25 50 100	1.9 4.4 3.6 1.7	16.6	4150	Y ⁶	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1991)
Aniline	MEK	10 25 50 100	1.7 7.7 7.5 1.5	13.3	3325	Y ⁷	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1991)
Anisyl alcohol	EtOH/DEP (1:3)	NA	NA	5.9	1475	N	NA	NA	(RIFM 2007)
A SC600	Pluronic L92	10 25 50 100	1.4 1.8 2.3 1.6	NC	NC	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)

³ Protocol used both sexes, and the test duration was 4 or 5 days.

⁴ Protocol used both sexes, and the test duration was 5 days.

⁵ Protocol used both sexes, and the test duration was 5 days.

⁶ Protocol used both sexes, and the test duration was 5 days.

⁷ Protocol used both sexes, and the test duration was 5 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Atrazine	ACE	10.0 20.0 30.0	1.3 1.4 0.8	NC	NC	Y ⁸	B6C3F1	Taconic Farms	(NTP 1994)
Atrazine	Pluronic L92	12.5 25.0 50.0 75.0 100.0	1.8 2.8 3.6 7.1 7.3	31.3	7813	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)
Atrazine	Pluronic L92	7.0 33.0 100.0	0.8 2.9 3.7	41.4	10344	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)
Basil oil	EtOH/DEP (1:3)	2.5 5 10 25 50	3 3 8 17.6 25.2	6.2	1550	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Benzalkonium chloride	ACE	0.5 1 2	9.0 11.1 7.6	0.1	17	Y ⁹	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
Benzocaine	AOO	NA	NA	37	9250	NA	NA	NA	(Griem et al. 2003) (Smith and Hotchkiss 2001)
Benzocaine	DMF	NA	NA	18	4500	NA	NA	NA	(Griem et al. 2003) (Smith and Hotchkiss 2001)
Benzocaine	AOO	1 5 25	1.3 1.8 2.9	NC	NC	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)

⁸ Mouse strain was not CBA.

⁹ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Benzocaine	AOO	NA	NA	22	5500	Y ¹⁰	BALB/c	National Institute of Public Health and the Environment Breeding Colony (RIVM), The Netherlands	(Van Och et al. 2000)
Benzocaine	AOO	5.0 10.0 20.0	4.5 7.2 7.6	3.1	775	Y ¹¹	CBA/Ca	NA	(Kimber et al. 1989)
Benzocaine	ACE	10 25 50	1.9 1.5 1.2	NC	NC	Y ¹²	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1995)
Benzocaine	DMF	2.5 5 10	1.4 2.3 2.1	NC	NC	Y ¹³	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1993)
Benzocaine	DMF	1 5 25	1.9 7.4 3	1.8	450	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Basketter et al. 1995)
Benzocaine	DMF	1 5 12.5 25	1.7 3.1 2.4 1.4	4.7	1175	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Basketter et al. 1995)
Benzocaine	DMF	1 5 12.5 25	1.4 1.4 2.2 1.5	NC	NC	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Basketter et al. 1995)

¹⁰ Mice were pretreated with SDS. Mouse strain was not CBA.

¹¹ LLNA study length was 3 days.

¹² Protocol used both sexes.

¹³ Protocol used both sexes.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 ($\mu\text{g}/\text{cm}^2$)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Benzocaine	DMF	1 5 12.5 25	1.6 1.5 2.4 1	NC	NC	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Basketter et al. 1995)
Benzocaine	DMF	5 10 12.5 15 25	3.2 2.4 3.6 1.8 2.4	4.54 ¹⁴	1135	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Basketter et al. 1995)
Benzoic acid	ACE	5 10 20	0.8 0.9 0.8	NC	NC	Y ¹⁵	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
Benzoisothiazolione	DMF	10 30 50	3.8 4.4 4.9	2.3	575	N	CBA	NA	(Ashby et al. 1995)
Benzoisothiazolione	NA	NA	NA	10.4	2600	N	CBA/Ca	NA	(Basketter et al. 1999d)
Benzoisothiazolione	DMF	3 10 30 50	1.56 1.22 2.79 4.53	32.4	8103	Y ¹⁶	CBA/Ca	NA	(Botham et al. 1991)
Benzoisothiazolione	DMF	3 10 30 50	2.72 3.84 4.45 4.97	4.8	1188	Y ¹⁷	CBA/Ca	NA	(Botham et al. 1991)

¹⁴ Interpolation of the EC3 was linear (per Ryan et al. 2007) and used concentration = 0% and SI = 1 as the lowest point.

¹⁵ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

¹⁶ Does not specify sex of mice used.

¹⁷ Does not specify sex of mice used.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Benzoquinone	AOO	0.5 1 2.5	36.4 42.3 52.3	0.0099	2.5	Y ¹⁸	CBA/Ca	NA	(Basketter and Scholes 1992)
Benzoyl peroxide	NA	NA	NA	0.30	75	NA	NA	NA	(Basketter and Kimber 2006)
Benzoyl peroxide	ACE	0.5 1 2.5 5 10	18.7 21 24.9 24.8 18.6	0.074	18	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
Benzoyl peroxide	ACE	0.5 1 2.5 5 10	14.6 17.2 18.1 20.2 21.8	0.023	5.8	Y ¹⁹	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)
Benzoyl peroxide	ACE	0.5 1 2.5 5 10	23.4 22.8 21.8 22.5 16.1	0.056	14	Y ²⁰	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)
Benzoyl peroxide	ACE	0.5 1 2.5 5 10	14.7 7.9 10.9 20.5 17.3	0.073	18	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)

¹⁸ Protocol used both sexes, and the test duration was 4 or 5 days.

¹⁹ Protocol used both sexes of mice.

²⁰ Protocol used both sexes of mice.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Benzoyl peroxide	ACE	0.5 1 2.5 5 10	24.4 22.1 33.7 31.4 26.5	0.043	11	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
Benzyl alcohol	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Benzylbenzoate	AOO	5 25	2.3 3.5	17.0	4250	NA	NA	NA	(Smith and Hotchkiss 2001)
Benzylbenzoate	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Benzyl cinnamate	EtOH/DEP (1:3)	NA	NA	18.4	4600	N	NA	NA	(RIFM 2007)
Benzylidene acetone	AOO	10 25 50	8.5 13.6 12.8	3.7	925	N	CBA/J	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Ryan et al. 2000)
Benzyl salicylate	EtOH/DEP (1:3)	NA	NA	2.9	725	N	NA	NA	(RIFM 2007)
Beryllium sulfate ²¹	DMSO	0.25 1 4	1.03 1.17 1.28	NC	NC	Y ²²	BALB/c	Charles River, Germany	(Mandervelt et al. 1997)
Beryllium sulfate	DMF	2.5 5 10	8.4 7.1 9.4	0.68	170	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Bourgeonal	EtOH/DEP (1:3)	NA	NA	4.30	1075	N	NA	NA	(RIFM 2007)

²¹ Data are for metal cation.

²² Mouse strain was not CBA.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
1-Butanol	Water	5 10 20	1.6 1.2 1.4	NC	NC	N	CBA/J	Harlan Sprague-Dawley, Inc., Indianapolis, IN or Jackson Laboratories, Bar Harbor, ME	(Ryan et al. 2000)
Butyl acrylate	AOO	1 2.5 5 10 25	0.7 1.3 1.5 2.5 8.7	11	2750	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2007)
Butyl glycidyl ether	AOO	10 25 50	1.4 2.2 5.6	30.9	7725	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Carvone	AOO	6 12 20	1.3 2.6 6.2	12.9	3225	N	CBA/Ca	Harlan, Horst, Netherlands	(Nilsson et al. 2005)
Carvone	AOO	NA	NA	13	3250	N	NA	NA	(RIFM 2007)
Carvone	EtOH/DEP (1:3)	NA	NA	10.7	2675	N	NA	NA	(RIFM 2007)
Carvone	EtOH/DEP (1:3)	NA	NA	5.7	1425	N	NA	NA	(RIFM 2007)
Chloroamine T	NA	NA	NA	0.4	100	NA	NA	NA	(Basketter and Kimber 2006)
4-Chloroaniline	NA	NA	NA	6.5	1625	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003b)
4-Chloroaniline	AOO	2.5 5 10	1.1 1.8 3.3	9	2250	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
4-Chloroaniline	AOO	2.5 5 10	2.1 1.6 2.5	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
4-Chloroaniline	AOO	2.5 5 10	1 1.5 1.8	NC	NC	N	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park, UK	(Scholes et al. 1992)
4-Chloroaniline	AOO	2.5 5 10	1 1.5 1.8	NC	NC	Y ²³	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter and Scholes 1992) (Scholes et al. 1992)
(Chloro)methylisothiazolinone	AOO	0.00375 0.0075 0.015 0.0375 0.075	1.3 2.6 7 10.9 14	0.0082	2.1	N	CBA	NA	(Ashby et al. 1995)
(Chloro)methylisothiazolinone	AOO	NA	NA	0.05	13	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003a)
(Chloro)methylisothiazolinone	AOO	0.00075 0.0015 0.0075 0.015 0.0375	0.9 1.2 4.4 9.1 8.5	0.0049	1.2	N	CBA/Ca	Harlan Seralab, Oxon, UK	(Warbrick et al. 1999a)
(Chloro)methylisothiazolinone	AOO	0.0075 0.015 0.038 0.075 0.15	1.5 4.4 7.8 10.8 9.5	0.01	2.5	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999c)
(Chloro)methylisothiazolinone	DMF	0.001 0.003 0.01 0.03 0.1	1.02 0.89 3.57 12.31 27.73	0.008	2.0	N	CBA/Ca	NA	(Botham et al. 1991)

²³ Protocol used both sexes, and the test duration was 4 or 5 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
(Chloro)methylisothiazolinone	DMF	0.0015 0.0075 0.015 0.0375 0.075	1.5 3 4.7 10.3 28	0.008	1.9	N	CBA/Ca	Harlan Seralab, Oxon, UK	(Warbrick et al. 1999a)
(Chloro)methylisothiazolinone	DMF	0.01 0.03 0.1	3.5 12.3 22.7	0.009	2.3	N	CBA	NA	(Ashby et al. 1995)
(Chloro)methylisothiazolinone	MEK	0.0015 0.0075 0.015 0.0375 0.075	0.9 3.3 8.4 14 17.6	0.007	1.7	N	CBA/Ca	Harlan Seralab, Oxon, UK	(Warbrick et al. 1999a)
(Chloro)methylisothiazolinone	DMSO	0.0015 0.0075 0.015 0.0375 0.075	1 3 9.5 6.4 10.3	0.008	1.9	N	CBA/Ca	Harlan Seralab, Oxon, UK	(Warbrick et al. 1999a)
(Chloro)methylisothiazolinone	PG	0.0015 0.0075 0.015 0.0375 0.075	2 0.8 2.1 2.3 4.7	0.048	12	N	CBA/Ca	Harlan Seralab, Oxon, UK	(Warbrick et al. 1999a)
(Chloro)methylisothiazolinone	PG	0.00375 0.0075 0.015 0.0375 0.075	0.8 0.8 0.8 1.5 3.7	0.063	16	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003a)

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 ($\mu\text{g}/\text{cm}^2$)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
(Chloro)methylisothiazolinone	ACE	0.005 0.05 0.1	8.1 27.8 48.2	0.003	0.69	Y ²⁴	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
(Chloro)methylisothiazolinone	ACE	0.0015 0.0075 0.015 0.0375 0.075	1.2 2.9 9.3 17.7 23.5	0.008	1.9	N	CBA/Ca	Harlan Seralab, Oxon, UK	(Warbrick et al. 1999a)
Chlorpromazine	DMF	0.25 2.5 25	1.02 1.75 3.53	18.3	4575	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Chlorpromazine	DMF	10 25 50	11.8 13.7 8.9	1.85 ²⁵	463 ²⁶	N	CBA/Ca	NA	(Basketter et al. 1994)
Cinnamic aldehyde	AOO	NA	NA	1.4	350	NA	NA	NA	(Smith and Hotchkiss 2001)
Cinnamic aldehyde	AOO	5 10 25	12.5 18.4 15.4	2.00	500	Y ²⁷	CBA/Ca	NA	(Basketter and Scholes 1992)
Cinnamic aldehyde	AOO	1 2.5 5	4.8 7.4 9.8	0.53	133	Y ²⁸	CBA/Ca	NA	(Kimber et al. 1989)

²⁴ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

²⁵ Interpolation of the EC3 was linear (per Ryan et al. 2007) and used concentration = 0% and SI = 1 as the lowest point.

²⁶ Schneider and Akkan (2004) report an EC3 of 463 $\mu\text{g}/\text{cm}^2$ from these data.

²⁷ Protocol used both sexes, and the test duration was 4 or 5 days.

²⁸ Does not specify sex of mice used.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Cinnamic aldehyde	AOO	0.5 1 2.5 5 10	1.4 0.9 1.9 7.1 15.8	3.1	775	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)
Cinnamic aldehyde	AOO	NA	NA	1.7	425	N	NA	NA	(Basketter et al. 2007)
Cinnamic aldehyde	AOO	NA	NA	2.7	675	N	NA	NA	(Basketter et al. 2007)
Cinnamic aldehyde	AOO	NA	NA	1.3	325	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Elahi et al. 2004)
Cinnamic aldehyde	AOO	NA	NA	2.2	550	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Elahi et al. 2004)
Cinnamic aldehyde	EtOH/DEP (3:1) + AO Mix	NA	NA	1.3	325	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	EtOH/DEP (3:1) + 0.1% TrlC	NA	NA	1.3	325	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	EtOH/DEP (3:1)	NA	NA	0.9	225	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	EtOH/DEP (3:1) + 0.1% Toc	NA	NA	1.1	275	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	DMSO	NA	NA	0.9	225	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Cinnamic aldehyde	EtOH/DEP (3:1) + 2% Toc	NA	NA	0.8	200	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	EtOH/DEP (3:1) + 0.1% TrlC	NA	NA	0.7	175	N	NA	NA	(RIFM 2007)

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Cinnamic aldehyde	EtOH/DEP (3:1) + AO Mix	NA	NA	0.7	175	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	EtOH/DEP (3:1) + 2% Toc	NA	NA	0.6	150	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	EtOH/DEP (3:1) + 0.1% Toc	NA	NA	0.2	50	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	EtOH/DEP (3:1)	NA	NA	0.2	50	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	AOO	1 2.5 5 10 25	2.2 3.9 4.6 7.6 5.4	1.7	426	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Cinnamic aldehyde	DMF	1 5 25	4.3 9.8 12.8	0.7	171	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Cinnamic aldehyde	DMF	0.25 0.5 1 2.5 5	1.5 3.1 4.5 8.3 8.6	0.5	116	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Cinnamic aldehyde	MEK	1 2.5 5 10 25	2.8 6.2 8.5 14.6 13.2	1.1	272	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Cinnamic aldehyde	PG	1 2.5 5 10 25	2.1 5.8 8.2 16.3 17	1.4	341	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Cinnamic aldehyde	DMSO	0.25 0.5 1 2.5 5	1.7 2.3 4.4 7.6 7.6	1.3	313	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Cinnamic aldehyde	EtOH (10%)	1 2.5 5 10 25	2.7 3.5 4.8 5.2 5.8	1.6	391	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Cinnamic aldehyde	EtOH (50%)	1 2.5 5 10 25	2.1 9.5 10.3 13.6 21.9	1.2	296	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Cinnamyl alcohol	AOO	10 25 50 90.0	1.8 3.5 3.9 5.7	21	5250	N	NA	NA	(Gerberick et al. 2005)
Cinnamyl alcohol	AOO	NA	NA	19.1	4775	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Elahi et al. 2004)
Cinnamyl nitrile	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
C.I. Reactive Red 231	AOO	1 3 9 15	4.8 3.4 4.4 4.6	0.6	150	N	CBA	NA	(Haist et al. 2007)
C.I. Reactive Yellow 174	AOO	1 3 9 15	4.2 5.3 5.5 7.8	0.3	75	N	CBA	NA	(Haist et al. 2007)
Citral	EtOH/DEP (3:1) + 0.1% Toc	NA	NA	6.8	1700	N	NA	NA	(RIFM 2007)
Citral	AOO	5 10 20	2.1 5 9.3	6.6	1638	Y ²⁹	CBA/Ca	Animal Breeding Unit, Environmental Safety Laboratory, Unilever Research	(Basketter et al. 1991; Basketter and Scholes 1992)
Citral	AOO	5 10 20	0.9 2.2 6.2	12.0	3000	Y ³⁰	CBA/Ca	Harlan Olac, Ltd., Oxon, UK	(Basketter et al. 1991)
Citral	AOO	5 10 20	2.2 8.1 20.5	5.7	1419	Y ³¹	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park, UK	(Basketter et al. 1991)
Citral	AOO	5 10 20	0.9 2.4 4.7	12.6	3152	Y ³²	CBA/Ca	Harlan Olac, Ltd., Oxon, UK	(Basketter et al. 1991)
Citral ³³	AOO	5 10 25	2.9 6.4 12.9	5.1	1275	N	CBA	NA	(Ashby et al. 1995)

²⁹ Protocol used both sexes.

³⁰ Protocol used both sexes.

³¹ Protocol used both sexes.

³² Protocol used both sexes.

³³ Referred to as citral PQ in Ashby et al. (1995).

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Citral	AOO	5 10 25	1.2 2.1 6.3	13	3250	Y ³⁴	NA	NA	(Gerberick et al. 2005)
Citral	EtOH/DEP (3:1)	NA	NA	5.3	1325	N	NA	NA	(RIFM 2007)
Citral	EtOH/DEP (1:3)	NA	NA	1.2	300	N	NA	NA	(RIFM 2007)
Citral	EtOH/DEP (1:3)	2.5 5 10 25 50	2.8 2.3 5.1 11.4 22.1	6.3	1575	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Citral	EtOH/DEP (3:1)	NA	NA	4.6	1150	N	NA	NA	(RIFM 2007)
Citral	EtOH/DEP (3:1) + 0.1% TrIC	NA	NA	5.8	1450	N	NA	NA	(RIFM 2007)
Citral	EtOH/DEP (3:1) + AO Mix	NA	NA	4.6	1150	N	NA	NA	(RIFM 2007)
Citral	EtOH/DEP (3:1) + 0.1% TrIC	NA	NA	3.7	925	N	NA	NA	(RIFM 2007)
Citral	EtOH/DEP (3:1) + AO Mix	NA	NA	2.1	525	N	NA	NA	(RIFM 2007)
Citral	EtOH/DEP (3:1) + 0.1% Toc	NA	NA	1.5	375	N	NA	NA	(RIFM 2007)

³⁴ Protocol used a 4-day exposure period and both sexes.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Citronella oil	EtOH/DEP (1:3)	2.5 5 10 25 50	1.4 0.9 1.2 1.2 2.7	NC	NC	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
dl-Citronellol	EtOH/DEP (1:3)	NA	NA	43.5	10875	N	NA	NA	(RIFM 2007)
Clove oil (bud)	EtOH/DEP (1:3)	1.0 2.5 5.0 10.0 25.0	1.1 1.8 2.5 3.7 5.9	7.1	1775	N	NA	NA	(RIFM 2007)
Clove oil (leaf)	EtOH/DEP (1:3)	2.5 5 10 25 50	1.6 1.5 4.0 9.5 11.4	7.1	1775	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Clove oil (stem)	EtOH/DEP (1:3)	1.0 2.5 5.0 10.0 25.0	1.6 1.7 2.2 4.2 8.9	7	1750	N	NA	NA	(RIFM 2007)
Cobalt (II) salts (cobalt chloride)	Water	0.5 1 2.5 5	2.08 3.51 3.77 7.21	0.8	200	Y ³⁵	CBA/N	Japan SLC Inc. Shizuoka, Japan	(Ikarashi et al. 1992)

³⁵ Test was terminated 24 hours after the last topical exposure.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Cobalt (II) salts (cobalt chloride)	DMSO	0.5 1 2.5	3.2 3.7 2.8	0.4	100	Y ³⁶	CBA/Ca	NA	(Basketter and Scholes 1992)
Cobalt (II) salts (cobalt chloride)	DMSO	1 2.5 5	1.5 1.6 2.7	NC	NC	Y	BALB/c	Charles River, Germany	(Mandervelt et al. 1997)
Copper (II) chloride	DMSO	1 2.5 5	8.1 13.8 13.6	0.4	100	Y ³⁷	CBA/Ca	NA	(Basketter and Scholes 1992; Basketter et al. 1999b)
Coumarin	AOO	5.0 10.0 25.0	2.7 2.9 2.3	NC	NC	N	NA	NA	(Gerberick et al. 2005)
Coumarin	DMF	10 25 50	0.9 2.05 3.2	45.7	11413	Y ³⁸	NA	Charles River, L'Arbresl, France	(Vocanson et al. 2006)
Coumarin	DMF	10 25 50	1.9 3.7 4	19.2	4792	Y ³⁹	NA	Charles River, L'Arbresl, France	(Vocanson et al. 2006)
Coumarin	DMF	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Cyclamen aldehyde	AOO	1 2.5 10 25	1.4 1.34 1.84 3.26	22.3	5575	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)
Damascone	AOO	NA	NA	1.24	310	N	NA	NA	(RIFM 2007)
Damascone	AOO	NA	NA	1.22	305	N	NA	NA	(RIFM 2007)
t-alpha Damascone	AOO	NA	NA	3.30	825	N	NA	NA	(RIFM 2007)

³⁶ Protocol used both sexes, and the test duration was 4 or 5 days.

³⁷ Protocol used both sexes, and the test duration was 4 or 5 days.

³⁸ Protocol used CBA/J or Balb/c mice.

³⁹ Protocol used CBA/J or Balb/c mice.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
trans beta Damascone	AOO	NA	NA	2.40	600	N	NA	NA	(RIFM 2007)
D EC 25	Pluronic L92	0.5 1 2.5	0.56 0.63 0.59	NC	NC	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
delta Damascone	AOO	NA	NA	0.866	217	N	NA	NA	(RIFM 2007)
delta Damascone	AOO	NA	NA	5.19	1298	N	NA	NA	(RIFM 2007)
delta Damascone	EtOH/DEP (1:3)	NA	NA	9.6	2400	N	NA	NA	(RIFM 2007)
D EW 15	Pluronic L92	2.5 5 10 25	1.9 1.5 2.5 2.5	NC	NC	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
Dextran	AOO	NA	NA	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003b)
1,2 Dibromo-2,4-dicyanobutane	AOO	0.5 1 2.5 5	1.4 3.4 3.5 5.4	0.9	225	N	NA	NA	(Gerberick et al. 2005)
1,2 Dibromo-2,4-dicyanobutane	AOO	NA	NA	1.3	325	N	NA	NA	(Basketter et al. 2007)
1,2 Dibromo-2,4-dicyanobutane	AOO	NA	NA	1.8	450	N	NA	NA	(Basketter et al. 2007)
1,2 Dibromo-2,4-dicyanobutane	AOO	NA	NA	5.2	1300	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003b)
1,2 Dibromo-2,4-dicyanobutane	NA	NA	NA	2.0	500	N	CBA/Ca	B&K Universal AB, Sollentuna, Sweden	(Basketter et al. 2005)
1,2 Dibromo-2,4-dicyanobutane	NA	NA	NA	2.3	575	NA	NA	NA	(Estrada et al. 2003)
Diethylenetriamine	AOO	5 10	6.4 12.1	3.3	825	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Diethylmaleate	AOO	25 50 100	16.3 22.6 13.1	5.8	1450	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2000)

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Diethylmaleate	AOO	NA	NA	2.1	525	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003b)
Diethylmaleate	NA	1 2.5 5 10 25	2.1 3.3 3.5 7.5 16	2	500	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999c)
Diethylmaleate	NA	NA	NA	4.7	1175	NA	NA	NA	(Estrada et al. 2003)
Diethyl phthalate	AOO	25 50 100	1.0 1.3 1.5	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2000)
Dihydrocoumarin	AOO	2.5 5 10	1.6 2.5 6.6	5.6	1400	N	CBA	NA	(Ashby et al. 1995)
Dihydrocoumarin	DMF	2.5 5 10	2.1 5.1 7	3.3	813	Y ⁴⁰	CBA/J or Balb/c	Charles River, L'Arbresl, France	(Vocanson et al. 2006)
1,4-Dihydroquinone	AOO	0.05 0.1 0.25 0.5 1.0	1.3 2.7 9.2 17.2 25.8	0.11	28	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Lea et al. 1999)
1,4-Dihydroquinone	AOO	0.05 0.1 0.25 0.5 1	1.3 1.2 4.3 11.2 12.1	0.19	48	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Lea et al. 1999)

⁴⁰ Protocol used CBA/J or Balb/c mice.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
1,4-Dihydroquinone	AOO	0.1 0.25 0.50 0.1 2.5	2.8 5.8 13.7 15.2 13.1	0.11	28	Y ⁴¹	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)
1,4-Dihydroquinone	DMF	0.05 0.1 0.25 0.5 1.0	1.6 1.8 3.2 7.7 10.9	0.23	58	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Lea et al. 1999)
1,4-Dihydroquinone	DMF	0.05 0.1 0.25 0.5 1	0.8 1.8 3.7 7.3 8	0.19	48	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Lea et al. 1999)
1,4-Dihydroquinone	MEK	0.05 0.1 0.25 0.5 1	1.9 2.9 13.9 23 24.5	0.10	25	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Lea et al. 1999)
1,4-Dihydroquinone	MEK	0.05 0.1 0.25 0.5 1.0	2.2 3.6 14.0 19.8 30.9	0.08	20	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Lea et al. 1999)

⁴¹ Protocol used both sexes of mice.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
1,4-Dihydroquinone	PG	0.05 0.1 0.25 0.5 1.0	0.7 0.9 1.2 1.9 2.0	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Lea et al. 1999)
1,4-Dihydroquinone	AOO	0.1 0.25 0.50 0.1 2.5	2.5 5.8 6.0 8.4 12.2	0.12	30	Y ⁴²	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)
1,4-Dihydroquinone	AOO	0.1 0.25 0.50 0.1 2.5	2.4 7.0 11.1 15.9 15.0	0.12	30	N	CBA/JHsd	Harlan Sprague- Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
1,4-Dihydroquinone	AOO	0.1 0.25 0.50 0.1 2.5	3.6 4.8 12.0 15.3 23.2	0.063	16	N	CBA/JHsd	Harlan Sprague- Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
1,4-Dihydroquinone	AOO	0.1 0.25 0.50 0.1 2.5	3.2 14.9 23.7 25.3 33.4	0.091	23	N	CBA/JHsd	Harlan Sprague Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
5,5-Dimethyl-3- methylenedihydro-2(3H)-furanone	AOO	2 4 8	3 7.4 9.2	1.8	450	N	CBA	NA	(Ashby et al. 1995)

⁴² Protocol used both sexes of mice.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Dimethyl sulfoxide	AOO	25 50 100	2.7 2.3 3.9	72	18000	NA	NA	NA	(Estrada et al. 2003)
2,4-Dinitrochlorobenzene	NA	NA	NA	0.10	25	N	NA	NA	(Estrada et al. 2003)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.1 0.25	1.5 1.8 2.4 8.9 38.0	0.048	12	Y ⁴³	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)
2,4-Dinitrochlorobenzene	AOO	0.01 0.05 0.10	6.2 15.7 24.0	0.0058	1.5	Y ⁴⁴	CBA/Ca	NA	(Basketter and Scholes 1992)
2,4-Dinitrochlorobenzene	DMSO	0.01 0.025 0.05 0.1 0.25	2.4 4.2 7.3 14.7 14.7	0.015	3.8	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Betts et al. 2006)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.1 0.25	1.4 2.20 4.00 9.8 16.2	0.036	9.0	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Betts et al. 2006)
2,4-Dinitrochlorobenzene	AOO	0.1 0.25 0.5	5.9 19.9 36.7	0.083	21	Y ⁴⁵	CBA/Ca	Animal Breeding Unit, Alderley Park, UK	(Kimber et al. 1991)

⁴³ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

⁴⁴ Protocol used both sexes, and the test duration was 4 or 5 days.

⁴⁵ Protocol did not specify sex, and the test duration was 4 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
2,4-Dinitrochlorobenzene	AOO	0.1 0.25 0.5	6.2 15.7 24.0	0.073	18	Y ⁴⁶	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Kimber et al. 1991)
2,4-Dinitrochlorobenzene	AOO	0.1 0.25 0.5	10.3 29.7 49.6	0.071	18	Y ⁴⁷	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
2,4-Dinitrochlorobenzene	AOO	0.1 0.25 0.5	4.7 15.8 26.8	0.087	22	Y ⁴⁸	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.10 0.25	2.0 2.3 5.3 10.5 35.5	0.027	6.8	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1995)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.10 0.25	0.8 1.8 3.3 8.7 40.9	0.046	12	N	CBA/J	Harlan Sprague Dawley Inc., Indianapolis, IN	(Kimber et al. 1995)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.10 0.25	1.1 1.4 2.5 4.6 11.5	0.062	16	Y ⁴⁹	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1995)

⁴⁶ Protocol did not specify sex, and the test duration was 4 days.

⁴⁷ Protocol did not specify sex, and the test duration was 4 days.

⁴⁸ Protocol did not specify sex, and the test duration was 4 days.

⁴⁹ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H- methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.1 0.25	0.8 1.2 1.7 3.1 22.5	0.094	24	N	CBA/J	Harlan Sprague Dawley Inc., Indianapolis, IN	(Kimber et al. 1995)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.1 0.25	1.3 1.5 2.1 7.7 43.9	0.057	14	Y ⁵⁰	CBA/J	Harlan Sprague Dawley Inc., Indianapolis, IN	(Kimber et al. 1995)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.1 0.25	1.5 1.9 3.1 6.5 25	0.05	13	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.1 0.25	1.2 0.9 2.9 4.5 13	0.06	15	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.1 0.25	2.5 2.9 3.2 7.1 25	0.033	8.3	Y ⁵¹	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)

⁵⁰ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H- methyl thymidine on the fifth day.

⁵¹ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.10 0.25	1.17 1.12 1.93 1.95 7.10	0.131	33	Y ⁵²	BALB/c	Charles River Laboratories	(NTP 1997a)
2,4-Dinitrochlorobenzene	ACE	0.001 0.05 0.1 0.25 0.5	0.8 10.7 21.1 2.2 1.8	0.012	3.0	Y ⁵³	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
2,4-Dinitrochlorobenzene	AOO	NA	NA	0.02	5.0	N	NA	NA	(Basketter et al. 2007)
2,4-Dinitrochlorobenzene	AOO	NA	NA	0.03	7.5	N	NA	NA	(Basketter et al. 2007)
2,4-Dinitrochlorobenzene	AOO	0.02 0.1 0.5	2 10.5 23	0.029	7.4	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
2,4-Dinitrochlorobenzene	AOO	NA	NA	0.044	11	Y ⁵⁴	Balb/c	National Institute of Public Health and the Environment Breeding Colony (RIVM), The Netherlands)	(Van Och et al. 2000)
2,4-Dinitrochlorobenzene	AOO	NA	NA	0.08	19	Y ⁵⁵	Balb/c	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1997)
Dinocap EC	Pluronic L92	0.8 4 21	2 14.2 26.7	1.1	266	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)

⁵² Mouse strain was not CBA.

⁵³ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

⁵⁴ Mice were pretreated with SDS. Mouse strain was not CBA.

⁵⁵ Mouse strain was not CBA.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Dinocap EC	Pluronic L92	0.8 4 21	2.23 25.77 14.38	0.9	226	N	CBA/Ca	NA	(ECPA 2007e)
Dinocap EC	Pluronic L92	0.8 4 20	1.3 11.5 15.6	1.3	333	N	CBA/J	R. Janvier, Le Genest St Isle, France	(ECPA 2007i)
Dinocap EC	Pluronic L92	0.8 4 10	1.3 4.08 10.94	2.8	689	N	CBA/JHsd	NA	(ECPA 2007g)
Dinocap EC	Pluronic L92	0.8 4 10	2.7 22.9 40.5	0.8	212	N	CBA/CaOla Hsd	NA	(ECPA 2006a)
Ethyl acrylate	AOO	NA	NA	28.7	7175	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Warbrick et al. 2001)
Ethyl acrylate	AOO	2.5 5 10 25 50	1.25 1.54 1.42 2.07 3.98	36.8	9200	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2007)
Ethylenediamine	AOO	0.5 1 2.5 5 10	1.6 1.9 3.3 6.1 17.4	2.20	550	Y ⁵⁶	NA	NA	(Kimber et al. 1998)
Ethylenediamine	ACE	1 5 10	1.1 1.1 2.2	NC	NC	Y ⁵⁷	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
Ethylenediamine	DMF	10	6.8	3.4	850	NA	NA	NA	(Akkan et al. 2003)

⁵⁶ The LLNA protocol used both sexes of mice.

⁵⁷ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Ethylenediamine	ACE/Water (3:1)	0.1 0.25 0.5 1 2.5	0.8 1.7 1.1 1.2 1	NC	NC	Y ⁵⁸	NA	NA	(Kimber et al. 1998)
Ethylene glycol dimethacrylate	NA	NA	NA	35.0	8750	NA	NA	NA	(Basketter and Kimber 2001)
Ethylene glycol dimethacrylate	NA	NA	NA	36.5	9125	NA	NA	NA	(Estrada et al. 2003)
Ethylene glycol dimethacrylate	MEK	10 25 50	1.2 2.4 7	28.0	7000	NA	NA	NA	(Gerberick et al. 2005)
Ethyl vanillin	AOO	2.5 5 10 25 50	0.65 1.05 0.74 0.36 0.29	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)
Eugenol	AOO	2.5 5 10 25 50	1.6 1.5 2.4 5.5 16	11.9	2975	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
Eugenol	AOO	2.5 5 10 25 50	1.1 1.7 1.8 9.1 12.4	8.9	2225	Y ⁵⁹	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)

⁵⁸ The LLNA protocol used both sexes of mice.

⁵⁹ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Eugenol	AOO	2.5	1.6	14.5	3625	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
		5	1.5						
		10	2.4						
		25	5.5						
		50	16						
Eugenol	AOO	25	4.8	18.9	4737	Y ⁶⁰	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Basketter and Scholes 1992); (Kimber et al. 1991)
		50	9.3						
		100	7.6						
Eugenol	AOO	25	7.2	9.5	2368	Y ⁶¹	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
		50	10.2						
		100	8.2						
Eugenol	AOO	25	5.5	20.4	5109	Y ⁶²	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
		50	14.1						
Eugenol	AOO	25	44.7	8.1	2021	Y ⁶³	CBA/Ca	Animal Breeding Unit, Alderley Park, UK	(Kimber et al. 1991)
		50	70.3						
		100	68.1						
Eugenol	AOO	25	1.2	40.9	10225	Y ⁶⁴	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park, UK	(Kimber and Weisenberger 1991)
		50	4						
Eugenol	AOO	2.5	2	5.8	1450	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
		5	2.8						
		10	3.2						
		25	13						
		50	17						

⁶⁰ Protocol did not specify sex, and the test duration was 4 days.

⁶¹ Protocol did not specify sex, and the test duration was 4 days.

⁶² Protocol did not specify sex, and the test duration was 4 days.

⁶³ Protocol did not specify sex, and the test duration was 4 days.

⁶⁴ Mice were exposed on flank by occluded patch prior to topical exposure on ears.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Eugenol	AOO	2.5	2.4	13.8	3450	Y ⁶⁵	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)
		5	2.1						
		10	1.2						
		25	5.3						
		50	9.6						
Eugenol	AOO	2.5	1.5	6.0	1500	N	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)
		5	4.3						
		10	4.6						
		25	14						
		50	6.1						
Eugenol	AOO	NA	NA	15.0	3750	N	NA	NA	(Basketter et al. 2007)
Eugenol	AOO	10	2.4	12.9	3225	N	CBA/Ca	NA	(Bertrand et al. 1997)
		25	5.5						
		50	16.1						
Eugenol	AOO	NA	NA	4.9	1225	N	NA	NA	(Basketter et al. 2007)
Eugenol	AOO	NA	NA	7.5	1875	N	NA	NA	(Basketter et al. 2007)
Eugenol	NA	NA	NA	13	3250	NA	NA	NA	(Basketter and Kimber 2001)
Eugenol	NA	NA	NA	11.6	2900	NA	NA	NA	(Kimber and Basketter 1997)
Eugenol	EtOH/DEP (1:3)	2.5	1.2	5.4	1350	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
		5	2.7						
		10	6						
		25	14.3						
		50	19.4						
Eugenol	ACE	25	5.4	18.2	4539	Y ⁶⁶	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
		50	10.6						
		75	10.5						

⁶⁵ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

⁶⁶ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Eugenol	EtOH/DEP (3:1)	NA	NA	5.3	1325	N	NA	NA	(Isola and Lalko 2001)
Eugenol	EtOH/DEP (1:3)	NA	NA	10.5	2625	N	NA	NA	(Isola and Lalko 2001)
Eugenol	EtOH	NA	NA	10.7	2675	N	NA	NA	(Isola and Lalko 2001)
Eugenol	DEP	NA	NA	15.1	3775	N	NA	NA	(Isola and Lalko 2001)
EXP 10810 A	Pluronic L92	10 25 50	6.4 8.4 9.2	2.1	527	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
EXP 11120 A	Pluronic L92	10 25 50 100	0.96 0.66 1.6 6.3	64.9	16223	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
FAR01042-00	Pluronic L92	10 25 50 100	1.4 2.1 1.4 2.5	NC	NC	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
FAR01060-00	Pluronic L92	10 25 50 100	0.4 0.8 1 3.6	88.5	22115	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
Farnesol	AOO	NA	NA	4.1	1025	N	NA	NA	(RIFM 2007)
Farnesol	AOO	NA	NA	5.5	1375	N	NA	NA	(RIFM 2007)
Fatty acid glutamate	NA	5 25 50 100	1.5 1.8 1.2 4.8	75.0	18750	N	NA	NA	(TNO 2006)

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Fatty alcohol #1	NA	10 25 50	4.2 8.2 16.2	7.6	1899	N	NA	NA	(TNO 2006)
Fatty alcohol #2	NA	10 25 50	4 9.9 16	8.6	2140	N	NA	NA	(TNO 2006)
F & Fo WG 50 + 25	Pluronic L92	2.5 5 10 25	11.7 12.6 14.1 15.2	0.003	0.77	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
Formaldehyde	ACE	0.25 0.5 1 2.5 5	NC NC NC NC 4	0.54 ⁶⁷	135	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Hilton et al. 1998)
Formaldehyde	ACE	0.093 0.185 0.37 0.925 1.85	1.1 2.3 2.3 3.9 4	0.61	153	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Hilton et al. 1998) [EC3]; (Gerberick et al. 2005) [Dose-response data]
Formaldehyde	ACE	NA	NA	0.65	163	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2005)
Formaldehyde	AOO	0.1 0.5 1 5 10	0.97 1.91 3.17 5.23 8.59	0.35	88	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)

⁶⁷ Hilton et al. (1998) report this EC3 as 0.18M.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Formaldehyde	AOO	5 10 25	9 10.6 11.9	0.37	93	Y ⁶⁸	CBA/Ca	Animal Breeding Unit, Alderley Park, UK	(Kimber et al. 1991)
Formaldehyde	AOO	NA	NA	0.40	100	NA	NA	NA	(Basketter and Kimber 2001)
Formaldehyde	AOO	0.093 0.185 0.37 0.925 1.85	1.1 2.3 2.3 3.9 4	0.70	175	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Hilton et al. 1998)
Formaldehyde	AOO	5 10 25	3.7 4 5.8	0.99	248	Y ⁶⁹	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Basketter and Scholes 1992; Kimber et al. 1991)
Formaldehyde	AOO	NA	NA	1.20	300	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Estrada et al. 2003)
Formaldehyde	DMF	1 10 20	6.7 13.2 17.7	0.27	67	N	CBA/Ca	Jackson Laboratories, Bar Harbor, ME	(Ryan et al. 2002)
Formaldehyde	DMF	0.25 0.5 1 2.5 5	NC NC NC NC >7	0.33 ⁷⁰	83	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Hilton et al. 1998)
Formaldehyde	AOO	5 10 25	6.8 6.1 6.6	1.72	430	Y ⁷¹	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)

⁶⁸ Protocol did not specify sex, and the test duration was 4 days.

⁶⁹ Protocol did not specify sex, and the test duration was 4 days.

⁷⁰ Hilton et al. (1998) report this EC3 as 0.11M.

⁷¹ Protocol did not specify sex, and the test duration was 4 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Formaldehyde	AOO	5 10 25	4.6 4.7 4.2	2.78 ⁷²	695	Y ⁷³	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
Formaldehyde	DMSO	1 10 20	7.5 16 17.6	0.30 ⁷⁴	74	N	CBA/Ca	Jackson Laboratories, Bar Harbor, ME	(Ryan et al. 2002)
Formaldehyde	Water	1 10 20	1.2 2.5 3.6	14.5	3636	N	CBA/Ca	Jackson Laboratories, Bar Harbor, ME	(Ryan et al. 2002)
Formaldehyde	PG	0.38 0.95 1.9 3.8 9.5	1.1 1.6 1.5 3.2 8.5	2.8	700	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003a)
Formaldehyde	Pluronic L92	1 5 20	1.1 3.8 10.6	3.8	950	N	CBA/Ca	NA	(ECPA 2007d)
Formaldehyde	Pluronic L92	1 5 20	1.6 2.6 12	5.6	1400	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)
Formaldehyde	Pluronic L92	1 5 20	0.99 2.16 6.15	8	2000	N	CBA/J	R. Janvier, Le Genest St Isle, France	(ECPA 2007i)
Formaldehyde	Pluronic L92	1 5 20	1.1 2.5 4.8	8.2	2050	N	CBA/JHsd	NA	(ECPA 2007j)

⁷² Interpolation of the EC3 was linear (per Ryan et al. 2007) and used concentration = 0% and SI = 1 as the lowest point.

⁷³ Protocol did not specify sex, and the test duration was 4 days.

⁷⁴ Interpolation of the EC3 was linear (per Ryan et al. 2007) and used concentration = 0% and SI = 1 as the lowest point.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Formaldehyde	Pluronic L92	1 5 20	0.8 1.3 4.8	12.3	3075	N	CBA/CaOla Hsd	NA	(ECPA 2006b)
Fumaric acid	DMSO	5 10 25	1.3 2.3 1.4	NC	NC	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borcheln	(EFfCI 2006)
Fx + Me EW 69	Pluronic L92	5 10 25 50	0.83 1.55 2.95 8.55	25.2	6306	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
Geraniol	EtOH/DEP (3:1)	1 3 10 30 50	1 1 1.3 3.4 3.9	25.8	6450	Y ⁷⁵	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko et al. 2004)
Geraniol	EtOH/DEP (1:3)	NA	NA	20.4	5100	Y ⁷⁶	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko et al. 2004)
Geraniol	EtOH/DEP (1:3)	2.5 5 10 25 50	1.7 2.4 2.8 4.8 6	11.4	2850	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Geraniol	AOO	NA	NA	57	14250	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon UK	(Griem et al. 2003)
Geraniol	AOO	12.5 25 50	0.9 1.2 2.6	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)

⁷⁵ Protocol used male mice.

⁷⁶ Protocol used male mice.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Geraniol	AOO	NA	NA	26	6500	N	NA	NA	(Roberts et al. 2007)
Geraniol	EtOH	NA	NA	5.6	1400	N	NA	NA	(Isola and Lalko 2001)
Geraniol	DEP	NA	NA	11.8	2950	N	NA	NA	(Isola and Lalko 2001)
Geranium oil	EtOH/DEP (1:3)	2.5 5 10 25 50	1.2 0.7 1.7 1.8 2.8	NC	NC	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Glutaraldehyde	AOO	NA	NA	0.2	50	NA	NA	NA	(Basketter et al. 2000)
Glutaraldehyde	AOO	0.039 0.052 0.13 0.26 0.52	1.6 2.4 4.9 5.1 11.3	0.07	18	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003a)
Glutaraldehyde	ACE	0.05 0.125 0.25 0.5 1.25	1.3 4.3 7.6 11.6 17.7	0.10	26	N	CBA/Ca	NA	(Gerberick et al. 2004)
Glutaraldehyde	ACE	NA	NA	0.09	23	NA	NA	NA	(Gerberick et al. 2001)
Glutaraldehyde	ACE	NA	NA	0.06	15	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Hilton et al. 1998)
Glutaraldehyde	DMF/Water	3.1 6.2 12.5	9.8 21.4 22.9	2.1	516	Y ⁷⁷	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)

⁷⁷ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Glutaraldehyde	DMF	0.25 0.5 1 2.5 5	>3 >3 >3 >3 >19	0.02	5.0	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Hilton et al. 1998)
Glutaraldehyde	PG	0.26 0.52 1.3 2.6	1 1.3 2.4 6.9	1.5	375	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003a)
Glycerol	DMF	25 50 100	1.1 0.7 0.5	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon UK	(Ryan et al. 2000)
Glyceryl thioglycollate	AOO	10 25 50	8 14 31	4.7	1165	N	NA	NA	(TNO 2006)
Glyoxal	DMF	5 10 25	18.1 13.5 12.2	0.6	150	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Glyoxal	AOO	1 2.5 5 10 25	2.5 4.2 5.2 10.3 15.8	1.4	350	NA	NA	NA	(Patlewicz et al. 2002)[EC3]; (Gerberick et al. 2005) (Dose-response data)
Glyoxal	NA	NA	NA	0.5	125	NA	NA	NA	(Basketter and Kimber 2006)
Gold chloride	DMSO	5 10 25	21.8 10.9 17.2	0.48 ⁷⁸	120	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999b)

⁷⁸ Interpolation of the EC3 was linear (per Ryan et al. 2007) and used concentration = 0% and SI = 1 as the lowest point.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Hexane	AOO	25 50 100	0.8 0.8 2.2	NC	NC	NA	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1996)
trans-2-Hexenal	AOO	0.5 1 2.5 5.0 10.0	1.2 1.2 2.3 2.6 6.4	5.5	1375	NA	NA	NA	(Patlewicz et al. 2002)[EC3]; (Gerberick et al. 2005) [Dose-response data]
trans-2-Hexenal	EtOH/DEP (1:3)	NA	NA	2.6	650	N	NA	NA	(RIFM 2007)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	2.2 3.2 7.1 13.9 17.6	4.4	1100	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999c)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.7 2.1 4.4 8.1 14.5	7.0	1750	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.1 2.2 4.4 9.8 20.0	7.00	1750	Y ⁷⁹	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)

⁷⁹ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Hexyl cinnamic aldehyde	AOO	2.5	1.3	7.60	1900	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
		5	1.5						
		10	4.4						
		25	8.8						
		50	16.0						
Hexyl cinnamic aldehyde	AOO	2.5	1.4	7.90	1975	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
		5	2.1						
		10	3.3						
		25	8.3						
		50	14.0						
Hexyl cinnamic aldehyde	AOO	NA	NA	8	2000	NA	NA	NA	(Basketter and Kimber 2001)
Hexyl cinnamic aldehyde	AOO	2.5	1.3	8.10	2025	N	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)
		5	1.3						
		10	4.2						
		25	8.8						
		50	17.0						
Hexyl cinnamic aldehyde	AOO	2.5	1.3	8.40	2100	Y ⁸⁰	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)
		5	1.1						
		10	2.5						
		25	10.0						
		50	17.0						
Hexyl cinnamic aldehyde	AOO	2.5	1.4	8.8	2200	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)
		5	2.1						
		10	3.3						
		25	8.4						
		50	14.0						

⁸⁰ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 ($\mu\text{g}/\text{cm}^2$)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Hexyl cinnamic aldehyde	AOO	10 25 50.0	3.2 6.0 10.0	9.40	2350	N	CBA	NA	(Ashby et al. 1995)
Hexyl cinnamic aldehyde	AOO	1 2.5 5 10 25	1.5 1.7 2.2 2.8 8.2	10.6	2650	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.30 1.10 2.50 10.40 17.0	11	2750	Y ⁸¹	CBA/Ca	Harlan Sprague-Dawley, Frederick, MD	(Dearman et al. 2001)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.7 2.1 2.4 7.2 14.1	11.9	2975	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1 1.4 2 8.7 11.6	11.5	2875	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999c)
Hexyl cinnamic aldehyde	AOO	5 10 25	1.6 2.5 6.8	11.70	2925	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)
Hexyl cinnamic aldehyde	AOO	5 10 25	1.4 2.7 5.3	11.70	2925	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)

⁸¹ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Hexyl cinnamic aldehyde	AOO	NA	NA	12.02	3005	NA	NA	NA	(Patlewicz et al. 2001)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.0 1.4 2.0 8.7 11.6	12.20	3050	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.0 1.4 2.0 8.7 11.6	12.20	3050	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.3 2.1 2.7 7.8 13.4	10.90	2725	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)
Hexyl cinnamic aldehyde	AOO	1 2.5 5 10 25	0.98 1 1.48 1.78 5.65	14.7	3682	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)
Hexyl cinnamic aldehyde	ACE	3 10 30	4.56 6.63 9.86	1.2	303	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borchen	(Gamer et al. 2008)
Hexyl cinnamic aldehyde	Pluronic L92	3 10 30	1.2 4.6 18	6.7	1675	N	CBA/Ca	NA	(ECPA 2007a)
Hexyl cinnamic aldehyde	Pluronic L92	3 10 30	1.9 4.2 9.2	7	1750	N	CBA/J	R. Janvier, Le Genest St Isle, France	(ECPA 2007i)

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Hexyl cinnamic aldehyde	Pluronic L92	3 10 30	1.9 2.2 10.3	12	3000	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)
Hexyl cinnamic aldehyde	Pluronic L92	3 10 30	1.1 2.5 15.6	10.8	2700	N	CBA/JHsd	NA	(ECPA 2007j)
Hexyl cinnamic aldehyde	Pluronic L92	3 10 30	1.3 2.2 4.3	17.6	4400	N	CBA/CaHs dRcc(SPF)	RCC Ltd, Laboratory Animal Service, CH-4414 Füllinsdorf / Switzerland	(ECPA 2007b)
2-Hexylidene cyclopentanone	EtOH/DEP (1:3)	NA	NA	2.40	600	N	NA	NA	(RIFM 2007)
Hexyl salicylate	EtOH/DEP (1:3)	NA	NA	0.18	45	N	NA	NA	(RIFM 2007)
Hydrocortisone	NA	2.5 5 10	0.3 0.1 0.06	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999a)
4-Hydroxybenzoic acid	DMSO	5 10 25	1.4 1.5 1.3	NC	NC	Y ⁸²	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter and Scholes 1992); (Scholes et al. 1992)
4-Hydroxybenzoic acid	AOO	2.5 5 10	0.4 0.8 0.6	NC	NC	N	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park. UK	(Scholes et al. 1992)
4-Hydroxybenzoic acid	AOO	5 10 25	0.9 1 0.9	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
4-Hydroxybenzoic acid	AOO	5 10 25	1.4 1.5 1.3	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)

⁸² Protocol used both sexes, and the test duration was 4 or 5 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Hydroxycitronellal	AOO	2.5 5 10 25 50	2.2 1 0.8 1.1 7.1	33.0	8250	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)
Hydroxycitronellal	AOO	NA	NA	20.0	5000	NA	NA	NA	(Basketter and Kimber 2001)
Hydroxycitronellal	AOO	NA	NA	25.0	6250	NA	NA	NA	(Estrada et al. 2003)
Hydroxycitronellal	AOO	25 50 100	3.6 5.9 8.5	21.0	5250	Y ⁸³	CBA/Ca	NA	(Basketter and Scholes 1992)
Hydroxycitronellal	AOO	10 25 50	1.7 3.2 6.7	23.0	5750	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Hydroxycitronellal	AOO	NA	NA	27.5	6875	N	NA	NA	(Basketter et al. 2007)
Hydroxycitronellal	AOO	NA	NA	22.4	5600	NA	NA	NA	(RIFM 2007)
Hydroxycitronellal	NA	NA	NA	25.3	6313	NA	NA	NA	(Patlewicz et al. 2002)
Hydroxycitronellal	DMF	1 5 25	1.3 2.1 3.4	18.8	4712	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Hydroxycitronellal	EtOH/DEP (1:3)	NA	NA	19.3	4825	N	NA	NA	(Isola and Lalko 2001)
Hydroxycitronellal	DEP	NA	NA	19.7	4925	N	NA	NA	(Isola and Lalko 2001)
Hydroxycitronellal	EtOH/DEP (3:1)	NA	NA	22.2	5550	N	NA	NA	(Isola and Lalko 2001)
Hydroxycitronellal	EtOH	NA	NA	26.4	6600	N	NA	NA	(Isola and Lalko 2001)

⁸³ Protocol used both sexes, and the test duration was 4 or 5 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
2-Hydroxyethyl acrylate	AOO	5 10 25	10.7 14.8 18.1	1.4	350	N	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park, UK	(Scholes et al. 1992)
2-Hydroxyethyl acrylate	AOO	10 25	9 8.2	6.25	1563	Y ⁸⁴	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter and Scholes 1992) (Scholes et al. 1992)
2-Hydroxyethyl acrylate	DMF	10 25 50	13.8 11 11.7	1.56 ⁸⁵	390	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
2-Hydroxypropyl methacrylate	AOO	10 25 50	1.1 1.2 1.3	NC	NC	Y ⁸⁶	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter and Scholes 1992) (Scholes et al. 1992)
2-Hydroxypropyl methacrylate	AOO	10 25 50	0.8 1 0.9	NC	NC	N	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park, UK	(Scholes et al. 1992)
2-Hydroxypropyl methacrylate	AOO	10 25 50	1 1.9 0.8	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
2-Hydroxypropyl methacrylate	DMF	10 25 50	1.4 0.7 0.9	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
Imidazolidinyl urea	DMF	10 25 50	1.7 3.1 5.5	24	6000	Y ⁸⁷	CBA/Ca	NA	(Basketter and Scholes 1992)
Isocyclemone E	EtOH/DEP (1:3)	NA	NA	25.14	6285	N	NA	NA	(RIFM 2007)

⁸⁴ Protocol used both sexes, and the test duration was 4 or 5 days.

⁸⁵ Interpolation of the EC3 was linear (per Ryan et al. 2007) and used concentration = 0% and SI = 1 as the lowest point.

⁸⁶ Protocol used both sexes, and the test duration was 4 or 5 days.

⁸⁷ Protocol used both sexes, and the test duration was 4 or 5 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Isocyclocitral	EtOH/DEP (1:3)	NA	NA	7.35	1838	N	NA	NA	(RIFM 2007)
Isocyclogeraniol	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	0.5 1 5	0.9 6.3 31.0	0.5	125	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	1.5 2.5 29.8	0.6	150	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	1.6 4.3 24.4	0.6	150	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	1.8 2.9 23.2	0.6	150	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	2.3 1.6 23.6	0.6	150	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	NA	NA	0.6	150	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	NA	NA	0.6	150	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	NA	NA	0.7	175	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	0.5 1 5	1.2 4.2 18.4	0.7	175	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	NA	NA	0.7	175	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	0.5 1 5	1.1 1.8 23.2	0.8	200	N	CBA/Ca	NA	(Basketter and Cadby 2004)

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Isoeugenol	AOO	0.5 1 5	1.5 2.6 19.2	0.8	200	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	1.6 2.2 19.0	0.8	200	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	NA	NA	0.8	200	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	NA	NA	0.9	225	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	0.5 1 5	0.7 2.3 13.8	1	250	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	1.3 2.2 13.1	1	250	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	0.8 1.6 14.1	1.1	275	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	1 1.1 12.4	1.2	300	N	NA	NA	(Gerberick et al. 2005)
Isoeugenol	AOO	NA	NA	1.2	300	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	NA	NA	1.2	300	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	NA	NA	1.2	300	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	NA	NA	1.3	325	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	2.5 5 10.0	9.9 17.0 29.5	1.3	319	Y ⁸⁸	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)

⁸⁸ Protocol did not specify sex, and the test duration was 4 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Isoeugenol	AOO	0.5 1 5	1.2 3.2 8.7	1.3	325	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.25 0.50 1 2.5 5	1.5 2.2 2.5 4.9 10.0	1.3	325	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
Isoeugenol	AOO	NA	NA	1.3	325	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	NA	NA	1.3	325	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	0.5	1.1	1.3	332	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	2.5 5 10.0	7.8 13.1 14.6	1.3	334	Y ⁸⁹	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
Isoeugenol	AOO	NA	NA	1.4	350	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	0.5 1 5	1.6 1.6 14.7	1.4	357	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	2.5 5 10.0	7.5 13.1 25.3	1.4	358	Y ⁹⁰	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Basketter and Scholes 1992); (Kimber et al. 1991)
Isoeugenol	AOO	NA	NA	1.5	375	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	0.5 1 5	1.3 3.3 14.7	1.5	375	N	CBA/Ca	NA	(Basketter and Cadby 2004)

⁸⁹ Protocol did not specify sex, and the test duration was 4 days.

⁹⁰ Protocol did not specify sex, and the test duration was 4 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Isoeugenol	AOO	0.5 1 5	1.6 2.2 7.5	1.6	400	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	2.0 1.4 7.6	1.6	400	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.25 0.50 1 2.5 5	1.2 1.7 2.6 4.3 11.0	1.6	400	N	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)
Isoeugenol	AOO	NA	NA	1.7	425	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	NA	NA	1.7	425	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	0.5 1 5	1.0 1.3 7.5	1.8	450	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	1.2 1.4 19.3	1.8	450	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.25 0.50 1 2.5 5	2.9 1.7 2.3 3.8 6.8	1.8	450	Y ⁹¹	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)
Isoeugenol	AOO	NA	NA	1.9	475	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	0.5 1 5	0.9 1.0 7.2	1.9	475	N	CBA/Ca	NA	(Basketter and Cadby 2004)

⁹¹ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Isoeugenol	AOO	0.5 1 5	1.4 1.2 6.7	2	500	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	0.8 2.8 5.6	2.1	525	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	2.5 5 10.0	4.2 11.8 21.3	2.2	560	Y ⁹²	CBA/Ca	Animal Breeding Unit, Alderley Park, UK	(Kimber et al. 1991)
Isoeugenol	AOO	NA	NA	2.3	575	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	NA	NA	2.3	575	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	0.5 1 5	1.4 1.5 4.9	2.6	650	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	1.7 1.2 5.0	2.6	650	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	NA	NA	2.7	675	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	NA	NA	2.8	700	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	NA	NA	2.9	725	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	0.25 0.50 1 2.5 5	0.7 0.7 0.9 2.1 7.2	3.1	775	Y ⁹³	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)

⁹² Protocol did not specify sex, and the test duration was 4 days.

⁹³ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Isoeugenol	AOO	0.25 0.50 1 2.5 5	1.0 1.3 2.1 2.3 4.1	3.3	825	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
Isoeugenol	AOO	0.5 1 2.5 5 10	1.8 2.9 7.7 11.1 11.7	1.0	258	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Isoeugenol	DMF	0.5 1 2.5 5 10	2.6 2.7 3.7 7.5 11.6	1.45	363	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Isoeugenol	DMSO	0.5 1 2.5 5 10	1.9 3.2 7.4 20 17.1	0.9	231	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Isoeugenol	EtOH (10%)	0.5 1 2.5 5 10	1.8 2 3.8 5.8 12.6	1.8	458	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Isoeugenol	EtOH (50%)	0.5 1 2.5 5 10	1 1.2 2 3 5.4	5.0	1250	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Isoeugenol	MEK	0.5 1 2.5 5 10	0.9 3.2 5 4.9 8.1	1.0	239	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Isoeugenol	NA	NA	NA	1.40	350	NA	NA	NA	(Kimber and Basketter 1997)
Isoeugenol	NA	NA	NA	3.50	875	Y ⁹⁴	NA	NA	(Estrada et al. 2003)
Isoeugenol	PG	0.5 1 2.5 5 10	0.8 1.6 3 5.3 8.5	2.5	625	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Isomethyl ionone	EtOH/DEP (1:3)	NA	NA	21.8	5450	N	NA	NA	(RIFM 2007)
Isopropanol	AOO	10 25 50	1.7 1.1 1.0	NC	NC	N	CBA	NA	(Basketter 1998)
Isopropyl myristate	AOO	25 50 100	2.1 3.3 3.4	44	11000	N	CBA/J	Harlan Sprague Dawley Inc., Indianapolis, IN	(Ryan et al. 2000)
Jasmine absolute (grandiflorum)	EtOH/DEP (1:3)	NA	NA	5.90	1475	N	NA	NA	(RIFM 2007)
Jasmine absolute (sambac)	EtOH/DEP (1:3)	NA	NA	36.40	9100	N	NA	NA	(RIFM 2007)
Kanamycin	AOO	5 10 25	2.2 0.8 1	NC	NC	NA	NA	NA	(Basketter et al. 1996)

⁹⁴ Protocol used both sexes of mice.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Lead acetate	DMSO	2.5 5 10	0.7 0.8 1	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999b)
Lemongrass oil	EtOH/DEP (1:3)	2.5 5 10 25 50	0.9 2.1 5.1 10.3 13.1	6.5	1625	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Lilial	AOO	1 2.5 5 10 25	1.3 2.47 NA 2.02 3.71	18.7	4675	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)
Lilial	AOO	NA	NA	16.8	4200	N	NA	NA	(RIFM 2007)
Lilial	EtOH	NA	NA	2.9	725	N	NA	NA	(RIFM 2007)
Lilial	DEP	NA	NA	4.1	1025	N	NA	NA	(RIFM 2007)
Lilial	EtOH/DEP (1:3)	NA	NA	13.9	3475	N	NA	NA	(RIFM 2007)
Lilial	EtOH/DEP (3:1)	NA	NA	8.8	2200	N	NA	NA	(RIFM 2007)
d-Limonene	DEP	NA	NA	31	7750	N	NA	NA	(RIFM 2007)
d-Limonene	DEP	NA	NA	63	15750	N	NA	NA	(RIFM 2007)
d-Limonene	AOO	25 50 100	1.8 2.4 4.0	69	17250	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Gerberick et al. 2005) [EC3]; (Warbrick et al. 2001)[Dose-response data]
d-Limonene	EtOH	NA	NA	10	2500	N	NA	NA	(RIFM 2007)
d-Limonene	EtOH/DEP (3:1)	NA	NA	22	5500	N	NA	NA	(RIFM 2007)
d-Limonene	EtOH/DEP (1:3)	NA	NA	38	9500	N	NA	NA	(RIFM 2007)

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Linalool	AOO	NA	NA	55	13750	N	NA	NA	(RIFM 2007)
Linoleic acid	AOO	10	1.5	14.1	3523	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borcheln	(EFfCI 2006)
Linolenic acid	AOO	10 25 50	3.1 9.3 10.3	9.9	2463	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borcheln	(EFfCI 2006)
Litsea cubeba oil	EtOH/DEP (1:3)	2.5 5 10 25 50	2 2.3 3.3 7.9 16	8.4	2100	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Lyrall HMPCC	AOO	1 2.5 5 10 25	0.6 0.7 0.6 1.3 4.9	17	4250	NA	NA	NA	(Patlewicz et al. 2002)[EC3] ; (Gerberick et al. 2005) [Dose-response data]
Lyrall HMPCC	AOO	NA	NA	17.1	4275	N	NA	NA	(RIFM 2007)
Majantal	AOO	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Maleic acid	DMSO	10 25 50	6.7 16.1 16.1	7.0	1743	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borcheln	(EFfCI 2006)
Manganese chloride	Petrolatum	5 10 25	1.10 0.60 1.00	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999b)
Menthadiene-7-methyl formate	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Menthadiene-7-methyl formate	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
2-Mercaptobenzothiazole	DMF	1 3 10	2.3 4.4 8.6	1.7	425	Y ⁹⁵	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Gerberick et al. 2005) [EC3]; (Basketter et al. 1993) [Dose-response data]
2-Mercaptobenzothiazole	DMF	10 25 50	4.5 4.6 5.5	5.7 ⁹⁶	1428	Y ⁹⁷	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter and Scholes 1992); (Scholes et al. 1992)
2-Mercaptobenzothiazole	DMF	10 25 50	5.2 9.1 4.8	6.0	1500	N	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park, UK	(Scholes et al. 1992)
2-Mercaptobenzothiazole	DMF	10 25 50	9.8 9.5 8.9	2.3	570	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
2-Mercaptobenzothiazole	DMF	10 25 50	10 10.8 8.1	2.2	555	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
2-Mercaptobenzothiazole	DMF	1 5 25	3 9.9 17.1	1	250	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Mercuric (II) chloride	AOO	5 10	19.9 11.8	0.4	98	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
4-Methoxyacetophenone	AOO	10 25 50	1.3 1.0 1.0	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Gerberick et al. 2005) [EC3]; (Ryan et al. 2000) [Dose-response data]
Methoxy dicyclopentadiene carboxaldehyde	AOO	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
2-Methoxy-4-methylphenol	AOO	NA	NA	5.80	1450	N	NA	NA	(RIFM 2007)

⁹⁵ Protocol used both sexes of mice.

⁹⁶ Interpolation of the EC3 was linear (per Ryan et al. 2007) and used concentration = 0% and SI = 1 as the lowest point.

⁹⁷ Protocol used both sexes, and the test duration was 4 or 5 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
4-Methylaminophenol sulfate	DMF	0.5 1 2.5	2.5 3.4 6.7	0.8	200	Y ⁹⁸	CBA/Ca	NA	(Gerberick et al. 2005) [EC3]; (Basketter and Scholes 1992) [Dose-response data]
Methylanisylidene acetone	AOO	10 25 50	3.5 10 26.1	9.3	2325	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2000)
alpha-Methyl cinnamic aldehyde	AOO	NA	NA	4.5	1125	N	NA	NA	(RIFM 2007)
6-Methylcoumarin	ACE	5 10 25	1.0 1.0 1.1	NC	NC	N	CBA	NA	(Ashby et al. 1995)
6-Methylcoumarin	ACE	5 10 25	1.2 0.9 0.8	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1991)
Methyl dodecanesulfonate	AOO	1 2.5 5	21.6 39.9 48.6	0.4	98	Y ⁹⁹	CBA/Ca	NA	(Gerberick et al. 2005) [EC3] (Basketter and Scholes 1992) [Dose-response data]
Methylhexanedione	AOO	25 50 100	2.9 6 14.3	26.0	6500	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Gerberick et al. 2005) [EC3]; (Ryan et al. 2000) [Dose-response data]
Methylhydrocinnamal	AOO	2.5 5 10 25 50	1.22 1.36 2.61 4.21 10.69	13.7	3425	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)
Methylhydrocinnamal	AOO	NA	NA	22.0	5500	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Griem et al. 2003)

⁹⁸ Protocol used both sexes, and the test duration was 4 or 5 days.

⁹⁹ Protocol used both sexes, and the test duration was 4 or 5 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Methylhydrocinnamal	DMF	25 50 100	3.6 9 16.4	23.1	5775	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2000)
Methylhydrocinnamal	NA	NA	NA	14	3500	NA	NA	NA	(Basketter and Kimber 2001)
Methylisothiazolinone	AOO	0.25 0.5 1.0 2.5 5.0	1.5 1.5 1.8 3.8 2.5	1.9	475	NA	NA	NA	(Estrada et al. 2003) [EC3]; (Gerberick et al. 2005) [Dose-response data]
Methylisothiazolinone	AOO	0.049 0.099 0.197 0.493 0.985	1.5 1.5 1.8 3.8 2.5	0.4	100	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003a)
Methyl methacrylate	ACE	10 30 50 75 100	1.5 2.3 2 4.4 7.3	60	15000	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Betts et al. 2006)
Methyl methacrylate	AOO	10 30 50 75 100	1.4 1.5 1.5 2.1 3.6	90	22500	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Betts et al. 2006)
Methyl 2-nonynoate	EtOH (80%)	5 10 20	10.4 17.7 24.4	2.5	625	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2000)
Methyl 2-octynoate	EtOH/DEP (1:3)	NA	NA	0.5	125	N	NA	NA	(RIFM 2007)

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Methyl salicylate	DMF	5 10 25	2.3 2.5 3	25.0	6250	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Methyl salicylate	MEK	5 10 25	2.5 2.5 7.5	11.5	2875	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Methyl salicylate	AOO	1 2.5 5 10 20	1.2 1.5 1.2 1.8 2.9	NC	NC	Y ¹⁰⁰	CBA/J	Harlan Sprague Dawley Inc., Indianapolis, IN	(Kimber et al. 1995)
Methyl salicylate	ACE	1 2.5 5	0.8 0.8 0.8	NC	NC	Y ¹⁰¹	CBA/J	NA	(Gerberick et al. 1992)
Methyl salicylate	AOO	1 2.5 5 10 20	1.1 1.4 1.4 1.4 2	NC	NC	Y ¹⁰²	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)
Methyl salicylate	AOO	1 2.5 5 10 20	1.8 2 1.5 2.2 1.8	NC	NC	Y ¹⁰³	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)

¹⁰⁰ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

¹⁰¹ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

¹⁰² The LLNA protocol used both sexes of mice.

¹⁰³ The LLNA protocol used both sexes of mice.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Methyl salicylate	AOO	1 2.5 5 10 20	1 1.1 1.6 1.4 0.9	NC	NC	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
Methyl salicylate	AOO	1 2.5 5 10 20	1.2 1.1 1.3 1.9 1.2	NC	NC	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
Methyl salicylate	AOO	1 2.5 5 10 20	1.1 1.4 1.2 1.2 0.9	NC	NC	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
NAVY 14 08 723	AOO	1 3 9 15	5.1 4.8 5.7 5.2	0.49	123	N	CBA	NA	(Haist et al. 2007)
Neomycin sulfate	EtOH (25%)	0.5 1 2	0.9 0.9 0.9	NC	NC	Y ¹⁰⁴	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
Neomycin sulfate	DMSO	5 10 25	1 0.9 1	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)

¹⁰⁴ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Nickel (II) salts (nickel chloride)	DMF	0.25 0.9 1 2.5 5	2 2.4 1.6 1.6 2.2	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2002)
Nickel (II) salts (nickel sulfate)	DMSO	0.25 0.5 1 2.5 5	1.3 1.4 1.4 1.8 3.1	4.8	1202	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2002)
Nickel (II) salts (nickel chloride)	DMSO	1 2.5 5	1.5 2.2 2.4	NC	NC	Y ¹⁰⁵	CBA/Ca	NA	(Basketter and Scholes 1992)
Nickel (II) salts (nickel sulfate)	DMSO/ Water (9:1)	2.5 5	2.19 2.46	NC	NC	Y ¹⁰⁶	CBA/N	Japan SLC Inc., Shizuoka, Japan	(Ikarashi et al. 1992)
Nickel (II) salts (nickel sulfate)	EtOH (30%)	2.5 5 10	1.3 2.6 6.6	5.5	1375	Y ¹⁰⁷	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
Nickel (II) salts (nickel sulfate)	Hydroxy- propyl cellulose in MeOH	2.5 5 10	0.8 1 2	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
Nickel (II) salts (nickel sulfate)	Pluronic L92	0.25 0.5 1 2.5 5	2 2.4 2.8 3 2.3	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2002)

¹⁰⁵ Protocol used both sexes, and the test duration was 4 or 5 days.

¹⁰⁶ Test was terminated 24 hours after the last topical exposure.

¹⁰⁷ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 ($\mu\text{g}/\text{cm}^2$)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Nickel (II) salts (nickel sulfate)	Hydroxyprop-yl cellulose in MeOH	2.5 5 10	1.4 1.2 1.2	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
Nickel (II) salts (nickel sulfate)	Hydroxy-propyl cellulose in MeOH	2.5 5 10	0.6 0.7 0.5	NC	NC	N	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park, UK	(Scholes et al. 1992)
Nickel (II) salts (nickel sulfate)	Hydroxy-propyl cellulose in MeOH	2.5 5 10	0.8 0.6 0.6	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
Nickel (II) salts (nickel sulfate)	DMSO	0.5 1 2.5	1.1 1.5 1.5	NC	NC	Y ¹⁰⁸	CBA/Ca	NA	(Basketter and Scholes 1992)
Oakmoss	EtOH/DEP (1:3)	NA	NA	3.8	950	N	NA	NA	(RIFM 2007)
Octanoic acid	AOO	10 25 50	0.7 1 1.6	NC	NC	N	CBA	NA	(Basketter 1998)
1-Octen-3-yl acetate	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Octinol	AOO	10 25 50	5.6 8.8 11.2	4.7	1187	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borcheln	(EFfCI 2006)
Oleic acid	NA	10 25 50	2.6 14.9 6.9	10.5	2622	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borcheln	(EFfCI 2006)
Oxalic acid	DMF	4 10 25	2.1 4.5 4.2	6.3	1563	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)

¹⁰⁸ Protocol used both sexes, and the test duration was 4 or 5 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Oxalic acid	DMF	4 10 25	4.4 4.5 5	2.4	588	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Oxazolone	ACE	0.0001 0.005 0.05	1.6 8.7 55.2	0.001	0.27	Y ¹⁰⁹	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
Oxazolone	AOO	0.0025 0.005 0.01 0.025 0.5	3.8 6.2 7.7 15 23	0.0007	0.18	N	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)
Oxazolone	AOO	0.0025 0.005 0.01 0.025 0.5	3.9 4.8 6 12 13	0.0014	0.35	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
Oxazolone	AOO	0.0025 0.005 0.01 0.025 0.05	3.4 4.4 4 5.9 8.9	0.002	0.50	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
Oxazolone	AOO	0.0025 0.005 0.01 0.025 0.5	4 6.9 16 40 59	0.0025	0.63	Y ¹¹⁰	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)

¹⁰⁹ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

¹¹⁰ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Oxazolone	AOO	0.0025 0.005 0.01 0.025 0.05	2.9 4.9 12 22 33	0.003	0.75	Y ¹¹¹	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Gerberick et al. 2005) [EC3]; (Loveless et al. 1996) [Dose-response data]
Oxazolone	AOO	0.1 0.25 0.5	19.4 24.2 32	0.0044	1.1	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Oxazolone	Not specified	NA	NA	0.013	3.3	NA	NA	NA	(Estrada et al. 2003)
Oxyfluorfen EC	Pluronic L92	NA	NA	NC	NC	N	CBA/Ca	NA	(ECPA 2007f)
Oxyfluorfen EC	Pluronic L92	1 7 33	0.81 1.42 4.91	18.8	4700	N	CBA/J	R. Janvier, Le Genest St Isle, France	(ECPA 2007i)
Oxyfluorfen EC	Pluronic L92	1 7 33	1.13 1.49 3.14	30.8	7700	N	CBA/JHsd	NA	(ECPA 2007g)
Oxyfluorfen EC	Pluronic L92	1 7 33	1.2 1.2 5.4	18.1	4525	N	CBA/CaOla Hsd	NA	(ECPA 2007h)
Palmarosa oil	EtOH/DEP (1:3)	2.5 5 10 25 50	1.1 2.1 3.1 3.6 5	9.6	2400	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Penicillin G	DMF	10 25 50	5.6 6.9 17	1.6	400	N	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park, UK	(Scholes et al. 1992)

¹¹¹ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Penicillin G	DMF	5	1.1	11.7	2933	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
		10	2.7						
		25	6.3						
		50	6.5						
Penicillin G	DMF	5	1	8.7	2165	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
		10	3.8						
		25	8.9						
Penicillin G	DMSO	2.5	1.0	31.3	7825	N	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Gerberick et al. 2005) [EC3]; (Kimber et al. 1998)
		5.0	1.0						
		10.0	1.4						
		25.0	2.1						
		50.0	6.6						
Penicillin G	DMSO	10	1.5	19.8	4946	Y ¹¹²	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter and Scholes 1992); (Scholes et al. 1992)
		25	3.8						
		50	8.9						
Penicillin G	DMSO	2.5	1.3	16.1	4025	N	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)
		5.0	1.7						
		10.0	1.9						
		25.0	4.0						
		50.0	4.6						
Penicillin G	DMSO	2.5	0.8	46.4	11600	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
		5.0	0.7						
		10.0	0.8						
		25.0	1.3						
		50.0	3.4						

¹¹² Protocol used both sexes, and the test duration was 4 or 5 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Penicillin G	DMSO	2.5 5.0 10.0 25.0 50.0	0.9 1.0 0.8 1.3 3.4	46.5	11625	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
Penicillin G	DMSO	2.5 5.0 10.0 25.0 50.0	0.6 0.8 1.3 1.9 3.6	41.1	10275	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
Penicillin G	DMSO	NA	NA	16.7	4175	NA	NA	NA	(Basketter et al. 2007)
Penicillin G	DMSO	NA	NA	17.9	4475	NA	NA	NA	(Basketter et al. 2007)
Pentachlorophenol	DMSO	10 25 50	2.1 3.5 5.4	20.0	5000	NA	NA	NA	(Gerberick et al. 2005) [EC3]; (Basketter et al. 1996) [Dose-response data]
Pentaerythritol triacrylate	ACE	0.005 0.01 0.05 0.1	1.19 0.92 1.68 2.43	NC	NC	Y ¹¹³	BALB/c	Charles River, Laboratories	(NTP 1997b)
Perillaldehyde	AOO	0.5 1.0 2.5 5.0	1.2 1.1 0.9 4.3	8.1	2025	NA	NA	NA	(Gerberick et al. 2005)
Perillaldehyde	AOO	NA	NA	7.8	1950	NA	NA	NA	(Basketter et al. 2007)

¹¹³ Mouse strain was not CBA.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Perillaldehyde	NA	NA	NA	7.95	1988	NA	NA	NA	(Estrada et al. 2003)
Peru balsam absolute	EtOH/DEP (1:3)	NA	NA	2.50	625	N	NA	NA	(RIFM 2007)
Phenyl benzoate	AOO	NA	NA	19.6	4900	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2005)
Phenyl benzoate	AOO	NA	NA	17.1	4263	NA	NA	NA	(Estrada et al. 2003)
Phenyl benzoate	AOO	1 2.5 5 10 25	2 6.4 9.3 8.7 11.1	1.2	300	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999c)
Phenyl benzoate	AOO	5 10.0 25	2.3 2.1 3.5	20	5000	N	NA	NA	(Gerberick et al. 2005)
Phenylacetaldehyde	AOO	1 2.5 5 10.0 25	0.7 1.8 7.8 8.8 19	3.0	750	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Gerberick et al. 2005) [EC3]; (Basketter et al. 2001) [Dose-response data]
Phenylacetaldehyde	AOO	NA	NA	4.7	1175	NA	NA	NA	(Basketter et al. 2002)
Phenylacetaldehyde	AOO	25 50 100	15.5 23.8 24.1	8.8	2200	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2000)
4-Phenylenediamine	AOO	2.5 5 10	18.6 20 37.4	0.001	0.28	Y ¹¹⁴	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
4-Phenylenediamine	AOO	0.4 2	10.4 16.3	0.05	13	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)

¹¹⁴ Protocol did not specify sex, and the test duration was 4 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
4-Phenylenediamine	AOO	0.05 0.1 0.25 0.5 1	2.6 4.7 10.3 15.5 14.2	0.06	15	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Warbrick et al. 1999b)
4-Phenylenediamine	AOO	0.05 0.1 0.25 0.5 1	2.2 4.2 13.73 20.77 25.28	0.07	18	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Warbrick et al. 1999b)
4-Phenylenediamine	AOO	NA	NA	0.08	20	N	CBA/Ca	NA	(Basketter et al. 2007)
4-Phenylenediamine	AOO	0.05 0.1 0.25 0.5 1	2 3.3 10.2 20.5 26.4	0.10	25	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Warbrick et al. 1999b)
4-Phenylenediamine	AOO	NA	NA	0.12	30	NA	NA	NA	(Basketter et al. 2007)
4-Phenylenediamine	AOO	0.01 0.025 0.05 0.1 0.25	0.9 1.5 1.3 1.9 7.1	0.13	33	Y ¹¹⁵	CBA/Ca	RCC Basel, Itingen, Switzerland	(White et al. 2006)
4-Phenylenediamine	AOO	NA	NA	0.14	35	NA	NA	NA	(Basketter et al. 2007)

¹¹⁵ Protocol used both sexes of mice.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
4-Phenylenediamine	AOO	0.05 0.1 0.25 0.5 1	1.59 2.62 5.64 9.51 9.44	0.15	38	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Warbrick et al. 1999b)
4-Phenylenediamine	AOO	0.05 0.1 0.25 0.5 1	1.9 2.3 4 5.7 6.6	0.16	40	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Gerberick et al. 2005) [EC3]; (Warbrick et al. 1999b) [Dose-response data]
4-Phenylenediamine	AOO	NA	NA	0.18	45	NA	NA	NA	(Basketter et al. 2007)
4-Phenylenediamine	AOO	0.05 0.1 0.25 0.5 1	1.1 2.2 3.5 7.6 4.6	0.20	50	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Warbrick et al. 1999b)
4-Phenylenediamine	AOO	2.5 5 10	21 26 75.3	0.2	52	Y ¹¹⁶	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
4-Phenylenediamine	AOO	2.5 5 10	12.8 16.5 23.3	0.4	100	Y ¹¹⁷	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Kimber et al. 1991); (Basketter and Scholes 1992)
4-Phenylenediamine	AOO	2.5 5	6.5 23.7	2.2	543	Y ¹¹⁸	CBA/Ca	Animal Breeding Unit, Alderley Park, UK	(Kimber et al. 1991)
4-Phenylenediamine	NA	NA	NA	0.29	73	NA	NA	NA	(Estrada et al. 2003)

¹¹⁶ Protocol did not specify sex, and the test duration was 4 days.

¹¹⁷ Protocol did not specify sex, and the test duration was 4 days.

¹¹⁸ Protocol did not specify sex, and the test duration was 4 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Phenylpropionaldehyde	AOO	NA	NA	6.3	1575	NA	NA	NA	(Schneider and Akkan 2004)
Phthalic anhydride	AOO	NA	NA	0.36	90	NA	NA	NA	(Basketter and Kimber 2006)
Potassium dichromate	DMF	0.025 0.05 0.1 0.25 0.5	2.9 4.3 9.1 15.1 22.6	0.33	83	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Ryan et al. 2002)
Potassium dichromate	DMSO	0.25 0.5	8.8 10.1	0.01	2.8	Y ¹¹⁹	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
Potassium dichromate	DMSO	0.1 0.25 0.5	3.5 10.2 10.4	0.03	7.5	Y ¹²⁰	CBA/Ca	NA	(Basketter and Scholes 1992)
Potassium dichromate	DMSO	0.025 0.05 0.1 0.25 0.5	1.4 2.5 9.5 25.9 10.1	0.05	13	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Ryan et al. 2002)
Potassium dichromate	DMSO	0.025 0.05 0.1 0.25 0.5	1.7 2.9 4.5 10.4 19.1	0.058	15	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1995)
Potassium dichromate	DMSO	0.1 0.25 0.5	7.9 22.6 33.6	0.07	18	Y ¹²¹	CBA/Ca	Animal Breeding Unit, Alderley Park, UK	(Kimber et al. 1991)

¹¹⁹ Protocol did not specify sex, and the test duration was 4 days.

¹²⁰ Protocol used both sexes, and the test duration was 4 or 5 days.

¹²¹ Protocol did not specify sex, and the test duration was 4 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Potassium dichromate	DMSO	0.025 0.05 0.1 0.25 0.5	1.6 1.4 3.8 5.3 16.1	0.08	20	Y ¹²²	CBA/J	Harlan Sprague Dawley Inc., Indianapolis, IN	(Gerberick et al. 2005) [EC3] ; (Kimber et al. 1995) [Dose-response data]
Potassium dichromate	DMSO	0.025 0.05 0.1 0.25 0.5	1.9 1.7 2.2 5.9 13	0.122	31	N	CBA/J	Harlan Sprague Dawley Inc., Indianapolis, IN	(Kimber et al. 1995)
Potassium dichromate	DMSO	0.025 0.05 0.1 0.25 0.5	1.2 2.1 3.4 4.5 11.2	0.132	33	Y ¹²³	CBA/J	Harlan Sprague Dawley Inc., Indianapolis, IN	(Kimber et al. 1995)
Potassium dichromate	DMSO	0.025 0.05 0.1 0.25 0.5	1.1 1.3 2.3 5.1 13.1	0.15	38	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1995)
Potassium dichromate	DMSO	0.1 0.25 0.5	1.8 5.1 6.9	0.15	39	Y ¹²⁴	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Kimber et al. 1991)
Potassium dichromate	DMSO	0.1 0.25 0.5	2.0 4.1 5.4	0.17	43	Y ¹²⁵	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)

¹²² LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

¹²³ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

¹²⁴ Protocol did not specify sex, and the test duration was 4 days.

¹²⁵ Protocol did not specify sex, and the test duration was 4 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Potassium dichromate	DMSO	0.5 1 2.5 5	2.12 3.07 4.01 3.8	0.96	240	Y ¹²⁶	CBA/N	Japan SLC Inc., Shizuoka, Japan	(Ikarashi et al. 1992)
Potassium dichromate	NA	NA	NA	0.1	25	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999c)
Potassium dichromate	Pluronic L92	0.02 0.1 0.5	2.4 2.9 7.9	0.11	28	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)
Potassium dichromate	Pluronic L92	0.025 0.05 0.1 0.25 0.5	1.1 1.1 1.4 4.9 5.4	0.17	42	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Ryan et al. 2002)
Potassium dichromate	Pluronic L92	0.02 0.1 0.5	1.4 1.8 7.8	0.18	45	N	CBA/JHsd	NA	(ECPA 2007j)
Potassium dichromate	Pluronic L92	0.02 0.1 0.5	1.06 1.04 5.55	0.3	75	N	CBA/J	R. Janvier, Le Genest St Isle, France	(ECPA 2007i)
Potassium dichromate	Pluronic L92	0.02 0.1 0.5	1.7 1.5 4.1	0.33	83	N	CBA/CaHs dRCC (SPF)	NA	(ECPA 2006d)
Produkt P-4G	AOO	1 3 9 15	2.4 2.5 1.9 2.5	NC	NC	N	CBA	NA	(Haist et al. 2007)
Propylene glycol	Water	50.0 100.0	1.2 1.6	NC	NC	N	CBA	NA	(Basketter 1998)

¹²⁶ Test was terminated 24 hours after the last topical exposure.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Propyl gallate	AOO	5 10 25	22.3 18.3 33.6	0.32	80	Y ¹²⁷	CBA/Ca	NA	(Basketter and Scholes 1992)
Propylidene phthalate	AOO	5 10.0 25	4.9 9.1 15.1	3.7	925	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2000)
Propylparaben	AOO	5 10.0 25	1.4 1.6 1.3	NC	NC	Y ¹²⁸	CBA/Ca	NA	(Basketter and Scholes 1992)
Pyridine	AOO	25 50 100	1.1 2.3 3.9	71.9	17975	NA	NA	NA	(Basketter et al. 1996)
Quinoxifen/cyproconazole	Pluronic L92	7 33 100	2.09 10.66 20.3	9.8	2440	N	CBA/Ca	NA	(ECPA 2007g)
Quinoxifen/cyproconazole	Pluronic L92	7 33 100	1.2 7.2 12.4	14.8	3700	N	CBA/J	R. Janvier, Le Genest St Isle, France	(ECPA 2007i)
Quinoxifen/cyproconazole	Pluronic L92	12.5 25 50 75 100	2 2.3 8.6 15.8 30.1	27.8	6944	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)
Quinoxifen/cyproconazole	Pluronic L92	7 33 100	0.4 3.8 2.0	26.9	6721	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)

¹²⁷ Protocol used both sexes, and the test duration was 4 or 5 days.

¹²⁸ Protocol used both sexes, and the test duration was 4 or 5 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Quinoxifen/cyproconazole	Pluronic L92	7 33 100	1.35 1.95 6.2	49.8	12438	N	CBA/JHsd	NA	(ECPA 2007g)
Quinoxifen/cyproconazole	Pluronic L92	7 33 100	1.3 6.5 13.6	15.5	3875	N	CBA/CaOla Hsd	NA	(ECPA 2007g)
Quinoxifen SC	Pluronic L92	7 33 100	1.1 1.7 0.8	NC	NC	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)
Resorcinol	AOO	1 2.5 5 10 25	1.8 2.3 2.6 6.3 10.1	5.5	1385	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2007)
Resorcinol	AOO	1 5 10 25 50	0.7 2.2 5.2 8.4 10.4	6.3	1583	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2007)
Resorcinol	AOO	0.1 0.25 0.5 1 2.5	0.4 0.2 0.5 0.8 1	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2007)
Resorcinol	DMF	5 10 25	2.2 2.2 2.7	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Salicylic acid	ACE	1 10 20	0.9 1.8 7.2	12.2	3056	Y ¹²⁹	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
Salicylic acid	AOO	5 10.0 25	0.8 1.5 2.5	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Sodium lauryl sulfate	DMF	1 2.5 5 10 20	2.7 4.2 4.6 8.9 8.6	1.5	375	Y ¹³⁰	CBA/JHsd	Harlan Sprague Dawley Inc., Frederick, MD	(Loveless et al. 1996)
Sodium lauryl sulfate	DMF	4 10 25	4.1 5.1 6.7	1.7	435	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Sodium lauryl sulfate	DMF	5 10 25	4 5.1 7.6	2.7	665	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Sodium lauryl sulfate	DMF	1 2.5 5 10 20	1.2 1.7 4.3 5.4 8	4.0	1000	N	CBA/JHsd	Harlan Sprague Dawley Inc., Frederick, MD	(Loveless et al. 1996)
Sodium lauryl sulfate	Pluronic L92	5 10 25	3.05 4.78 8.46	4.9	1225	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borcheln	(Gamer et al. 2008)

¹²⁹ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

¹³⁰ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 ($\mu\text{g}/\text{cm}^2$)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Sodium lauryl sulfate	DMF	1	1.5	4.4	1100	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
		2.5	2.3						
		5	3.8						
		10	4.1						
		20	5.3						
Sodium lauryl sulfate	DMF	1	0.9	13.4	3350	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
		2.5	1.1						
		5	1.7						
		10	2.6						
		20	3.5						
Sodium lauryl sulfate	DMF	1	1.6	17.1	4275	Y ¹³¹	CBA/JHsd	Harlan Sprague Dawley Inc., Frederick, MD	(Loveless et al. 1996)
		2.5	2.1						
		5	2.8						
		10	1.6						
		20	3.6						
Sodium lauryl sulfate	DMSO	5	3.5	2.5	625	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
		10	4						
		25	4.2						
Sodium lauryl sulfate	DMSO	5	3.2	3.1	773	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Gerberick et al. 2005) [EC3] (Basketter et al. 1996) [Dose-response data]
		10	4						
		25	4.2						
Spearment oil	EtOH/DEP (1:3)	0.5	1.2	8.2	2050	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
		1	1.1						
		2.5	1.2						
		5	1.9						
		10	3.6						

¹³¹ LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ³H-methyl thymidine on the fifth day.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Squalene	AOO	10 25 50	3.8 6.9 8.2	7.9	1975	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borcheln	(EFfCI 2006)
Streptomycin	DMF	2.5 5 10.0 25 50	1.4 1.6 2.1 2.9 3.2	33	8250	Y ¹³²	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)
Streptomycin	DMF	2.5 5 10.0 25 50	1.2 1.4 1.3 2 1.9	NC	NC	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
Streptomycin	DMF	2.5 5.0 10.0 25.0 50.0	1.3 1.2 1 1.2 1.3	NC	NC	Y ¹³³	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)
Streptomycin	DMF	2.5 5.0 10.0 25.0 50.0	1.7 0.8 0.6 1.1 1.2	NC	NC	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
Streptomycin	DMF	2.5 5.0 10.0 25.0 50.0	1 0.8 0.9 1.1 1.3	NC	NC	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)

¹³² The LLNA protocol used both sexes of mice.

¹³³ The LLNA protocol used both sexes of mice.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Succinic acid	DMSO	5 10 25	1.2 1.2 1.3	NC	NC	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borcheln	(EFfCI 2006)
Sulfanilamide	DMF	10.0 25.0 50.0	1.0 1 0.9	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Sulfanilic acid	DMF	5 10 25	1.5 1.9 2.2	NC	NC	Y ¹³⁴	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1992)
Sulfanilic acid	DMF	5 10 25	1.1 1.2 1.3	NC	NC	Y ¹³⁵	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1992)
Sulfanilic acid	DMF	5 10 25	1.9 1.2 1.8	NC	NC	Y ¹³⁶	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1992)
Sulfanilic acid	DMSO	2.5 5 10	1.3 1.3 1.5	NC	NC	Y ¹³⁷	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1992)
Tartaric acid	DMF	5 10 25	1 0.9 1.5	NC	NC	N	NA	NA	(Gerberick et al. 2005)
Tea leaf absolute	DMF	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Tetrachlorosalicylanilide	ACE	0.25 0.5 1	11.2 14.4 18	0.04	10	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)

¹³⁴ Protocol used both sexes.

¹³⁵ Protocol used both sexes.

¹³⁶ Protocol used both sexes.

¹³⁷ Protocol used both sexes, and the test duration was 4 days.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm ²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Tetrachlorosalicylanilide	ACE	0.1 0.25 0.5	16 27.8 40.5	0.03	7.8	N	CBA/Ca	Harlan Olac Ltd.	(Scholes et al. 1991)
Tetramethylthiuramdisulphide	AOO	2.5 5 10.0	2.4 2.9 5.1	5.2	1300	NA	NA	NA	(Basketter et al. 1996)
Tetramethylthiuramdisulphide	AOO	NA	NA	6.0	1500	NA	NA	NA	(Basketter and Kimber 2001)
Thioglycerol	DMF	10 25 50	6.7 10 10	3.6	895	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Toluene 2,4-diisocyanate	AOO	NA	NA	0.1	28	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003b)
Treemoss	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Trifluralin EC	Pluronic L92	7 33 100	5.96 30.04 75.24	5.8	1446	N	CBA/Ca	NA	(ECPA 2007c)
Trifluralin EC	Pluronic L92	7 33 100	1.9 8.7 25.7	11.2	2801	N	CBA/J	R. Janvier, Le Genest St Isle, France	(ECPA 2007i)
Trifluralin EC	Pluronic L92	7 33 100	3.1 26.3 61.5	7.0	1738	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)
Trifluralin EC	Pluronic L92	7 33 100	1.03 6.98 16.12	15.6	3902	N	CBA/JHsd	NA	(ECPA 2007g)
Trifluralin EC	Pluronic L92	7 33 100	1.8 8.2 20.5	11.9	2969	N	CBA/CaOla Hsd	NA	(ECPA 2007k)

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 ($\mu\text{g}/\text{cm}^2$)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Trimellitic anhydride	NA	NA	NA	0.22	55	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003b)
Tween 80	AOO	NA	NA	NC	NC	NA	NA	NA	(Basketter et al. 2000)
Undec-10-enal	AOO	5.0 10.0 25.0 50.0 75.0	1.7 5.3 7.5 8.7 8.8	6.8	1700	NA	NA	NA	(Patlewicz et al. 2002)
Undecylenic acid	AOO	10 25 50	2.5 3.3 4.4	19.4	4844	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borcheln	(EFfCI 2006)
Vanillin	AOO	2.5 5 10.0 25 50	0.9 1.4 1.5 1.2 1.4	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)
Xylene	AOO	NA	NA	95.8	23950	NA	NA	NA	(Estrada et al. 2003)
YELLOW E-JD 3442	AOO	1 3 9 15	1 0.8 0.9 0.9	NC	NC	N	CBA	NA	(Haist et al. 2007)
Ylang Ylang	EtOH/DEP (1:3)	NA	NA	6.80	1700	N	NA	NA	(RIFM 2007)
Zinc sulfate	DMSO	5 10 25	1.3 2 2.3	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999b)

Abbreviations: ACE = acetone; AO Mix = antioxidant mixture of 0.3% butylated hydroxytoluene/tocopherol/eugenol (0.1% each); AOO = acetone: olive oil (1:4 by volume); Conc. = concentration; DEP = diethylphthalate; DMF = dimethylformamide; DMSO = dimethylsulfoxide; EtOH = ethanol; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay; MEK = methyl ethyl ketone; N = no; NA = not available; NC = not calculated because $SI < 3$; Nonstd. = nonstandard; Pet. = petrolatum; PG = propylene glycol; SI = stimulation index; Toc = tocopherol; TrIC = Trolox C; UK = United Kingdom; Y = yes.

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linda.katz@fda.hhs.gov

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Monday, November 30, 2015 10:25 AM
To: Katz, Linda
Subject: FW: ICSB 2016 invitation

Dear Linda

Hope you had good Thanksgiving. This looks more complicated than I thought. Any suggestion what to do next
IK

From: "Laurent.Selles@ec.europa.eu" <Laurent.Selles@ec.europa.eu>
Date: Monday, November 30, 2015 at 9:19 AM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: Ikhlas Khan <ICSB@olemiss.edu>, "linda.katz@fda.hhs.gov" <linda.katz@fda.hhs.gov>, "Petra.CADOVA@ec.europa.eu" <Petra.CADOVA@ec.europa.eu>
Subject: RE: ICSB 2016 invitation

(Dear Dr Khan) dear Ikhlas,

First of all I apologize for answering with some delay to your mail here-below.

Since our 'Health Technology and Cosmetics' Unit does not have the kind of competence you look for a presentation at the next ICSB Conference in April 2016, I inquired with colleagues working for other departments of the European Commission. These attempts were difficult because the whole European Commission is in the middle of re-organizations. Then I was (b) (6) for a week.

To seek clarification on the exact nature of the conference, I looked on the conference website. It would appear that the Directorate General DG "SANTE" would be the right place to identify a speaker competent in the field.

If this conference deals more with newly imported botanicals and their safety and approval, this could fall under Novel Foods and thus the new DG SANTE Unit E2 "Food processing technology and novel foods" led by M. IGLESIA. When it comes to botanicals being used in supplements or 'botanical claims', then DG SANTE F3 "Plants and Organisms" is the place.

If it's in relation to pesticides, residues, then the competence is with DG SANTE E4 "Pesticides and Biocides".

Would you please clarify what kind of speaker/ presentation you are looking for? I will try to help.

Best regards

Laurent

Laurent SELLES

Senior Coordinator for International Relations



European Commission

DG for Internal Market, Industry, Entrepreneurship and SMEs

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Tuesday, November 10, 2015 4:43 PM
To: SELLES Laurent (GROW)
Cc: icsb@olemiss.edu
Subject: ICSB 2016 invitation

Dear Laurent

DR. Linda katz at US FDA has referred your name to us. I am Associate Director at National Center for Natural products Research at University of Mississippi. As part of FDA cooperative agreement with us we organize annual conference on Science of Botanicals every year. We discuss science and policy around botanicals and we get international participation from Many countries including. Last few years we have introduced session on cosmetics and botanicals. We would like to invite some one who can talk about safety, quality/ regulation from European perspective.

I need you assistance to identify the speaker.

I will be happy to provide further information if needed.

Ik

Ikhlas A. Khan, Ph.D, D. Litt (Hon. Causa)
Associate Director, NCNPR
Director , FDA Center of Excellence
Director Center for Research in Indian Systems of Medicine (CRISM)
Director of Sino-US TCM Research Center
Research Professor Professor, Dept. of Pharmacognosy
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<http://www.pharmacy.olemiss.edu/ncnpr/index.html>

Tsai, Victoria

From: CRISTINA AVONTO <cavonto@olemiss.edu>
Sent: Wednesday, July 15, 2015 10:44 AM
To: Ikhlas Khan; Sadrieh, Nakissa; Katz, Linda
Cc: Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA
Subject: RE: list
Attachments: avonto_ICSB_FINAL.pdf

Dear Dr. Sadrieh,

Please find attached the ICSB presentation on the current status of chemical methods for skin sensitization. The two complementary techniques we developed are based on the same rationale as cys-DPRA, in other words we are able to identify and characterize potential sensitizers (as pure compounds) based on their electrophilic potential. One of the advantages is the applicability of the methods to qualitative evaluate mixtures (e.g. plant extracts, see chamomile and tea tree results), which is not possible with other chemical methods. Among other applications, the two DCYA methods can be very useful to rapidly identify unknown reactive substances of concern in mixtures for further characterization. Please let me know if we can provide more information or if you have any questions.

Regards

Cristina Avonto, PhD
Post Doctoral Research associate
NCNPR, School of Pharmacy
University of Mississippi, MS 38677
Phone: 662-9151027

From: Ikhlas Khan
Sent: Monday, July 13, 2015 2:20 PM
To: Sadrieh, Nakissa; Katz, Linda
Cc: Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: list

Dear Nakissa

Good to hear from you. LLNA and DPRA were used in our studies but sample were tested outside as. To screening botanicals and pure compounds, we have developed two assays which are included in the presentations we sent earlier.

H-CLAT is not used yet since its going under validation and checking the reproducibility before testing real compounds.

Cristina has given presentation at ICSB about new screens and presented Pros and Cos of these assays.

She will send the presentation to you soon.

Amar will be back next week and we will discuss about assays.

Please let us know if you have further questions.

IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Monday, July 13, 2015 at 11:59 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>, "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Cc: "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Subject: RE: list

Dear Dr. Khan,

Thank you for the information that you have sent us. Can I please ask for a clarification? In your slides, you mention that U Miss has the H-CLAT assay (Human Cell Line Activation Test) up and running, however, in previous briefings from Diego Rua who worked for OCAC, we were also told that the following assays had been used in work conducted for OCAC, by U Miss:

1. LLNA= Local lymph node assay
2. EASA = Electrophilic Allergen Screening Assay
3. DPRA = Direct peptide reactivity assay

Are the above 3 assays also available at U Miss? Please provide me with a description (the basis for the assay and what is measured) of all the available in vitro sensitization assays available at U Miss, as well as the pros and cons of these assays, meaning what are their strengths and weaknesses. This will help us in deciding which of these assays we may wish to use, and for which projects. Also, please let me know of other in vitro assays that you are aware of, for sensitization assessment, that we might want to look into, even if U Miss does not currently have these assay up and running. Specifically, please look at the link below, and the list provided in Table 1, of other methods that might be useful for the assessment of cosmetic product sensitizers. Thank you.

<http://www.sciencedirect.com/science/article/pii/S0273230015001415#t0005>

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Friday, July 10, 2015 11:10 AM

To: Katz, Linda

Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO

Subject: Re: list

Dear Linda

Attached, please find separate ppts to describe the results and approaches. Some slides might need further explanation, we can setup a call to go through over these slides if needed.
In the meantime anyone has a question, please feel free to contact us.
Have a nice weekend
IK

And publications

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Monday, June 29, 2015 at 12:45 PM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>
Subject: RE: list

Thanks very much!

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Monday, June 29, 2015 1:04 PM
To: Katz, Linda
Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: list

Dear Linda
We will send the information soon.
IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Monday, June 29, 2015 at 10:48 AM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>
Subject: RE: list

Ikhlas,

As you know there have been a number of personnel changes in OCAC. In order for us to plan our next steps and discuss further research needs including and beyond the allergens, it would be helpful for all of those involved in OCAC to be on the same page.

Please provide a summary of the in vitro sensitization assays available and the ingredients tested at your facility as well as the methods that will be used to assess the allergens of current concern. In addition, it would be helpful if you could provide us with a summary of the work done on arbutins, focusing on the scientific questions asked, methods developed, experiments performed and the results/conclusions. Nakissa will be working on our collaborative research projects for the interim and she may have additional questions for you. Thanks again for your help. (Your response needs not be extensive.)

Linda

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Monday, June 29, 2015 10:55 AM

To: Katz, Linda
Cc: Hansen, Patricia A; Sadrieh, Nakissa; Milstein, Stanley R
Subject: Re: list

Dear Linda

Thanks for sharing the list and the related information. We will collect information about the listed compounds and will be ready to discuss next steps soon.

Thanks
IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Thursday, June 25, 2015 at 9:47 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: "patricia.hansen@fda.hhs.gov" <patricia.hansen@fda.hhs.gov>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>
Subject: FW: list

Ikhlas,

Attached is the list of the 26 EU fragrance allergens as well as the SCCS opinion. Some of these substances are either confirmed contact allergens or presumptive contact allergens (but not confirmed); other tables present those which are suspected to be human contact allergens or which are based on animal, in-vitro (LLNA), or QSAR/*in-silico* data. Table 13-5 presents 12 established chemical fragrance allergens with high risk of sensitization to humans. In the SCCS Opinion, the Threshold exposure (for single chemicals only, not extracts or mixtures) which could be tolerated by most consumers is estimated to be $\leq 0.8 \mu\text{g}/\text{cm}^2$.

Chemical of special concern include: tree moss, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), oak moss, isoeugenol, hydroxycitronellal, citral, cinnamal, farnesol and cinnamyl alcohol. However, we are interested in the remainder of those listed below from Table 13-5.

Cinnamal
Cinnamyl Alcohol*
Citral
Coumarin
Eugenol*
Farnesol*
Geraniol*
Hydroxycitronellal
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)
Isoeugenol*
Limonene (oxidised)
Linalool* (oxidised)
*including their respective esters

The 2012 SCCS Opinion also recommends that fragrance pre-haptens and pro-haptens of several terpene fragrance materials should be considered as putative "allergens" and regulated in the same way as the allergens by the EC (Among them are limonene, linalool, linalyl acetate, geraniol, geranial, α -terpinene, eugenol, isoeugenol, and cinnamyl alcohol).

Let me know if you need any further information at this time regarding allergens.

On a different note, let me know if you have a report and list with description of in vitro assays, including validation, that actually describes the methods that you have developed for arbutin.

Linda

From: Hansen, Patricia A
Sent: Wednesday, June 17, 2015 1:21 PM
To: Ikhlas Khan
Cc: Katz, Linda
Subject: RE: list

Hi, Ikhlas. Sorry not to get back to you sooner.
I think the conversations we had while on our visit were very helpful.
By copy of this note, I'll alert Linda to your request for the fragrance allergen list. We talked about it during our visit and she may have assigned it to someone already, but I'm not sure.
Is there a conference call already scheduled, perhaps with the dietary supplement group and others or are you talking about something else?
Hope all is well.
PH

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Friday, June 05, 2015 10:58 AM
To: Hansen, Patricia A
Subject: list

Hi Pat
I know this is your first week and must be busy. Could you please share the list of 26 and may be 80 if possible.
It will help to us to see what is coming but you will still have time to prioritize before we have conference call
Thanks
IK

Tsai, Victoria

From: Amar Chittiboyina <amar@olemiss.edu>
Sent: Monday, August 17, 2015 11:38 AM
To: Sadrieh, Nakissa; 'Ikhlas Khan'; Katz, Linda
Cc: Milstein, Stanley R
Subject: RE: list
Attachments: 2015_SitevisitOCAC.pdf; 2015_CRT_NMR.pdf; LlNa-pot_3b_appc_brd_annexii-1.pdf

Dear Nakissa,

Thank you and your team for hosting us. The meeting was very productive and we are looking forward to work with you on OCAC needs on botanicals in cosmetics including 26 ingredients.

A copy of Dr. Khan's presentation (in PDF) is attached along with recently accepted publication on NMR spectroscopy.

Can you pass the following information to Raj also?

1. LLNA data on coumarin was one of the test article in the document on "Comparative LLNA, Guinea Pig, and Human Data Used in the Performance Evaluation". Please refer page #23 for coumarin data (attached).

ntp.niehs.nih.gov/iccvam/docs/immunotox_docs/lina-pot/3b-appc-brd-annexii-1.pdf

Please let us know if you need any additional information.

Sincerely
Amar

From: Sadrieh, Nakissa [mailto:Nakissa.Sadrieh@fda.hhs.gov]
Sent: Friday, July 17, 2015 10:58 AM
To: 'Ikhlas Khan'; Katz, Linda
Cc: Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Larry Walker
Subject: RE: list

We are trying to schedule the meeting between 2 and 4 pm. Is that OK with you or do you prefer from 10 am to 12pm?

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Friday, July 17, 2015 11:37 AM
To: Sadrieh, Nakissa; Katz, Linda
Cc: Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Larry Walker
Subject: Re: list

Hi Nakissa

Good, My presentation should cover all the topics yo suggested and after an overview we can discuss future plan.
We will plan to book return flight on 13th evening so we have enough time.
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, July 17, 2015 at 10:20 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>, "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Cc: "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>, Larry Walker <walker@olemiss.edu>
Subject: RE: list

Ikhlas,

It would be nice to get an overview that focuses on the work that the University of Mississippi has done to date on 1) botanicals, and specifically the arbutins, and 2) in vitro and in chemico methods that are available at U Miss, for screening of cosmetic sensitizers. I would like perhaps some slides included on potential areas of future work, based on our interests, which are covered by the 2 points indicated above. Please keep in mind that any work is to be directly linked to a regulatory outcome, and this is where I would probably provide guidance. Thanks.

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Friday, July 17, 2015 11:05 AM
To: Katz, Linda

Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Larry Walker

Subject: Re: list

Dear Linda

Thanks for quick response. Our meeting will end on 12th evening, we can stay next day and have meeting on 13th and return in the afternoon or evening back.

We have provided ppts but if you think it will be useful to give an overview, I can present the same presentation which I gave during your visit.

Nakissa, please let me know if you have some thoughts about the agenda or you would like to cover any specific topic

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Friday, July 17, 2015 at 7:24 AM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>

Subject: RE: list

Ikhlas,

I will be out of the office when you are here in August. However, Nakissa and Stan would love to meet with you and will probably also invite our colleagues in OARSA, who are doing related research, to attend for a broader discussion of methods and direction. Let me know your actual availability, including the amount of time you will have to spend with us so that we can make arrangements for a room. Also let me know if you will be making any formal presentations. We will work on an agenda from our end which we will share. Because I will be out of the office from August 3 through August 13, Nakissa will be your point of contact.

Linda

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Thursday, July 16, 2015 12:15 PM

To: Katz, Linda

Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO

Subject: Re: list

Dear Linda

During your visit we discussed a possibility to meet the OCAC group during our visit to CFSAN in August. We have COE meeting on August 12th. We can plan to meet on 13th if its feasible.

Let me know if we would like to meet and we will make our return travel plan accordingly.

ik

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Friday, July 10, 2015 at 10:45 AM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>

Subject: RE: list

Ikhlas,

Thanks so much for this information. We will be back in touch.

Have a nice weekend as well.

Linda

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Friday, July 10, 2015 11:10 AM
To: Katz, Linda
Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: list

Dear Linda

Attached, please find separate ppts to describe the results and approaches. Some slides might need further explanation, we can setup a call to go through over these slides if needed.

In the meantime anyone has a question, please feel free to contact us.

Have a nice weekend

IK

And publications

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Monday, June 29, 2015 at 12:45 PM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>
Subject: RE: list

Thanks very much!

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Monday, June 29, 2015 1:04 PM
To: Katz, Linda
Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: list

Dear Linda

We will send the information soon.

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Monday, June 29, 2015 at 10:48 AM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>
Subject: RE: list

Ikhlas,

As you know there have been a number of personnel changes in OCAC. In order for us to plan our next steps and discuss further research needs including and beyond the allergens, it would be helpful for all of those involved in OCAC to be on the same page.

Please provide a summary of the in vitro sensitization assays available and the ingredients tested at your facility as well as the methods that will be used to assess the allergens of current concern. In addition, it would be helpful if you could provide us with a summary of the work done on arbutins, focusing on the scientific questions asked, methods developed, experiments performed and the results/conclusions. Nakissa will be working on our collaborative research projects for the interim and she may have additional questions for you. Thanks again for your help. (Your response needs not be extensive.)

Linda

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Monday, June 29, 2015 10:55 AM
To: Katz, Linda
Cc: Hansen, Patricia A; Sadrieh, Nakissa; Milstein, Stanley R
Subject: Re: list

Dear Linda

Thanks for sharing the list and the related information. We will collect information about the listed compounds and will be ready to discuss next steps soon.

Thanks

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Thursday, June 25, 2015 at 9:47 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: "patricia.hansen@fda.hhs.gov" <patricia.hansen@fda.hhs.gov>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>
Subject: FW: list

Ikhlas,

Attached is the list of the 26 EU fragrance allergens as well as the SCCS opinion. Some of these substances are either confirmed contact allergens or presumptive contact allergens (but not confirmed); other tables present those which are suspected to be human contact allergens or which are based on animal, in-vitro (LLNA), or QSAR/*in-silico* data. Table 13-5 presents 12 established chemical fragrance allergens with high risk of sensitization to humans. In the SCCS Opinion, the Threshold exposure (for single chemicals only, not extracts or mixtures) which could be tolerated by most consumers is estimated to be $\leq 0.8 \mu\text{g}/\text{cm}^2$.

Chemical of special concern include: tree moss, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), oak moss, isoeugenol, hydroxycitronellal, citral, cinnamal, farnesol and cinnamyl alcohol. However, we are interested in the remainder of those listed below from Table 13-5.

Cinnamal
Cinnamyl Alcohol*
Citral
Coumarin
Eugenol*
Farnesol*
Geraniol*
Hydroxycitronellal
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)
Isoeugenol*

Limonene (oxidised)
Linalool* (oxidised)
*including their respective esters

The 2012 SCCS Opinion also recommends that fragrance pre-haptens and pro-haptens of several terpene fragrance materials should be considered as putative “allergens” and regulated in the same way as the allergens by the EC (Among them are limonene, linalool, linalyl acetate, geraniol, geranial, α -terpinene, eugenol, isoeugenol, and cinnamyl alcohol).

Let me know if you need any further information at this time regarding allergens.

On a different note, let me know if you have a report and list with description of in vitro assays, including validation, that actually describes the methods that you have developed for arbutin.

Linda

From: Hansen, Patricia A
Sent: Wednesday, June 17, 2015 1:21 PM
To: Ikhlas Khan
Cc: Katz, Linda
Subject: RE: list

Hi, Ikhlas. Sorry not to get back to you sooner.

I think the conversations we had while on our visit were very helpful.

By copy of this note, I'll alert Linda to your request for the fragrance allergen list. We talked about it during our visit and she may have assigned it to someone already, but I'm not sure.

Is there a conference call already scheduled, perhaps with the dietary supplement group and others or are you talking about something else?

Hope all is well.

PH

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Friday, June 05, 2015 10:58 AM
To: Hansen, Patricia A
Subject: list

Hi Pat

I know this is your first week and must be busy. Could you please share the list of 26 and may be 80 if possible.

It will help to us to see what is coming but you will still have time to prioritize before we have conference call

Thanks

IK

Tsai, Victoria

From: Ikhlas Khan <ikhan@olemiss.edu>
Sent: Friday, July 10, 2015 11:10 AM
To: Katz, Linda
Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: list
Attachments: inchemico methods_case studies.pptx; FDA_summary in vitro assays-v2.pptx; ARBUTIN summary.pptx; Arbutin_stability.pdf; 14123 Wang_Y.pdf

Dear Linda

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Thanks

IK

Risk of sensitization to fragrances estimated on the basis of patch test data and exposure, according to volume used and a sample of 5451 cosmetic products[†]

Axel Schnuch,^{a*} Wolfgang Uter,^b Holger Lessmann^a and Johannes Geier^a

ABSTRACT: The risk of sensitization cannot be derived from the frequency of sensitization to allergens alone, as exposure also should be considered. The occurrence of 26 fragrances in 5451 products based on the labelling of the ingredients was documented. Use volumes were provided by the International Fragrance Association (IFRA). Frequency of sensitization to fragrances was analysed based on IVDK data from September 2007 to December 2009. As an estimate of sensitization risk, the sensitization exposure quotient (SEQ) was calculated as the quotient of the relative frequency of sensitization and the relative frequency of use/labelling. The SEQs (the risk) varied greatly, offering a ranking regarding risk of sensitization: oak moss, tree moss, farnesol, methyl 2-octynoate (methyl heptene carbonate) and isoeugenol at the top of the list indicating a (very) high risk of sensitization, butylphenyl methylpropional (Lilial®), hexyl cinnamal, citronellol, linalool and limonene at the bottom, indicating a (very) low risk of sensitization. Compounds with a high risk were found to be classified as potent allergens according to the LLNA. High frequencies of sensitization may be put into perspective by the frequent use of certain fragrances. Despite infrequent use, others (with higher potencies or too high use concentrations) may turn out to be associated with an increased risk. Hazard assessment should be supplemented by risk assessment. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: fragrances; contact allergy; risk assessment; clinical epidemiology

Certain fragrance compounds must be considered important contact allergens, particularly those contained in the Fragrance Mixes.^[1,2] Over the past decades, further fragrances were identified as sensitizers.^[3–5] Fragrances differ with regard to frequency of sensitization,^[6,7] which had entailed a 'differentiated look' on the issue of fragrances as allergens.^[8]

The absolute frequencies of sensitization to allergens observed in large samples of patch test patients (and extrapolated on a population level^[9]) reflect their impact as contact allergens in absolute terms indicating a threat to the health of the population, or otherwise.

However, the question whether substance A poses a lower allergenic risk than substance B, which should therefore be replaced in products by A, cannot be answered. Such risk assessment must also take into account exposure data, i.e. relate the absolute risk to the frequency of exposure, thus arriving at a 'relative incidence'^[10] or, more generally, at a 'relative risk'.

In a former study, it was possible to relate extrapolated annual frequencies of sensitization (incidence) to topical drugs in the general population^[11] to the amount of drugs sold [defined daily doses (DDDs) per year], and calculate a 'relative incidence' (RI).^[10] For example, sensitization to gentamicin was more prevalent than sensitization to kanamycin (2077 vs. 1366 cases with periorbital dermatitis owing to the ingredient, per year, on the population level), but prescription and thus exposure via ophthalmological drugs in terms of DDDs was about three times higher for gentamicin than for kanamycin (43.3 vs. 15.4 mio DDD p.a.). Thus, the ranking of sensitization risk was actually reversed: the relative incidence (cases/100 000 DDD-year) for gentamicin was 4.8 and

for kanamycin 8.7. Thus exposure to kanamycin turned out to be associated with a greater risk of sensitization.^[10]

In another approach, using the sensitization exposure quotient (SEQ), which relates the sensitization frequencies of preservatives (as % positives of patients tested) to the use of preservatives as expressed by the frequency of occurrence on the labels of 3541 leave-on products documented by the CVUA Karlsruhe, we estimated the risk of sensitization. High frequencies of sensitization (e.g. to parabens) were put into perspective by their frequent use, resulting in a very low sensitization risk associated with parabens.^[12]

With regard to fragrances, it is only known that the 26 fragrances to be labelled according to current EU legislation ('26 EU fragrances') are used more or less often: Amyl cinnamal, benzyl benzoate, benzyl salicylate, butyl phenyl methyl propional, citral, citronellol, coumarin, eugenol, geraniol, hexyl cinnamal,

* Correspondence to: Axel Schnuch, Information Network of Departments of Dermatology (IVDK), University Medicine Göttingen, Von Siebold Str. 3, 37075 Göttingen, Germany. E mail: aschnuch@gwdg.de

[†] This article is part of the virtual special issue of the Flavour and Fragrance Journal entitled "Allergens in Flavour and Fragrance" edited by Professor Dr. med. Axel Schnuch and Dr. Alain Chaintreau.

^a Information Network of Departments of Dermatology, Georg August University, 37075 Göttingen, Germany

^b Department of Medical Informatics, Biometry and Epidemiology, University of Erlangen/Nürnberg, 91054 Erlangen, Germany

hydroxyisohexyl 3-cyclohexene carboxyaldehyde (HICC), alpha-isomethyl ionone and linalool are used in volumes greater than 175 tonnes, and amylcinnamyl alcohol, anise alcohol, benzyl alcohol, benzyl cinnamate, cinnamal, cinnamyl alcohol, farnesol, hydroxycitronellal, isoeugenol, *d*-limonene, methyl-2-octynoate, oak moss (*Evernia prunastri*) and tree moss (*Evernia furfuracea*) are used in volumes less than 175 tonnes.^[7]

Apart from such rough indications, only sparse data were published on exposure frequencies.^[13–16] However, the data set containing information on labelling used for the SEQs of preservatives mentioned above can also be used for fragrances. Furthermore, the volume of fragrances used in the year 2008 was provided by the International Fragrance association (IFRA). Thus two different sources of information on exposure to fragrances were available for analyses, in addition to the three smaller studies on labelling.^[13–15] The most recent study^[16] was not considered for analysis because it covered a different time period.

In this study, we analysed the frequency of sensitization to 26 fragrances to be labelled according to current EU legislation ('26 EU fragrances') and related sensitization to data on exposure as indicated by volume and labelling.

Methods

Assessment of exposure to fragrances

Total use volume

IFRA kindly provided us with the quantity (in tonnes) of the 26 single fragrance materials relevant here, sold for use in cosmetics Europe-wide in 2008. The total amount (the sum of all volumes of single fragrances) was set at 100%, and the share of single fragrances (in %) (i.e. the volume of a single fragrance related to the total of volumes) was calculated.

Fragrances contained in cosmetics

The labelling of cosmetic products ($n=5451$) purchased at random between 2007 and 2009 was documented by the CVUA (Chemisches und Veterinär-Untersuchungsamt Karlsruhe/Germany), aggregated into 24 product classes. The total products (5451) and leave-on products ($n=3541$) were considered for analysis. 15500 and 9568 occurrences of fragrances were documented, respectively. Thus a product contained 2.8 fragrance compounds on average. As the frequency of occurrence of a fragrance is regarded as indicator of actual exposure, it is used as the basis for further analysis.

The total frequency of use of fragrances ($n=15\,500$ and $n=9568$, respectively) was set at 100% and the relative frequency of use of single fragrances was calculated yielding the thus standardized share of products (%) containing the respective fragrance (Table 4, col. 5, and Table 5, col. 3 and 5)

Data on labelled cosmetics ($n=516$) put together from different smaller studies were used for similar calculations of the SEQ. There were 1800 occurrences of single fragrances, 3.5 times on the products on average (Table 6).^[13] Exposure to fragrances through different product categories (total: $n=300$) and more specifically to deodorants ($n=88$) was also assessed by documenting labelling.^[14,15] There were 5.9 and 6.4 labelled fragrances on average.

In all these labelling statistics, a distinction between high and low volume products was not possible. With a few high volume products the labelling based estimate of exposure would underestimate actual exposure (see comments below)

Frequency of sensitization

Data on sensitization frequency generated by the IVDK network^[17] between 2007 and 2009 and specifically 2008 were considered for specific analyses (For a more detailed description of the methods used by the IVDK see Geier *et al.* (this issue)^[18]).

To characterize the study population, the factors of the MOAHLFA-Index are given in Table 1 where M stands for the proportion of men, O for the proportion of occupational dermatitis, A for the proportion of patients with atopic dermatitis, H with hand dermatitis (H), L with leg dermatitis, F with face dermatitis (F) and A being at least 40 years old.

Results of patch testing FM I and FM II in the standard series and the results of break down testing in mix positives for the period 1 September 2007 to 31 December 31 2009 were analysed. During this period, a specific fragrance series (Almirall/Hermal, 'further fragrances') containing those of the '26 EU' fragrances not covered by FM I and FM II were applied in 1870 patients (in 2008: $n=823$). These were defined as the study population further considered. The proportion of reactions to single constituents in breakdown testing in mix positives from testing the standard series was extrapolated to the study population ($n=1870$) yielding the frequency of sensitization to single constituents.

The relative frequency of sensitization was calculated as the share of sensitization to a single allergen (%) relative to the total of sensitization (=100%) to fragrances (Table 4, column 3).

Sensitization exposure quotient (SEQ)

As an estimate of sensitization risk associated with exposure to the respective fragrance the SEQ is calculated as the quotient of the relative frequency of sensitization divided by the relative frequency of use (Table 4, column 6).

Results

Frequencies of sensitization

The results of breakdown testing in FM I positives [and sorbitan sesquioleate (SSO) negatives] ($n=806$) and FM II positives ($n=324$) from all patients tested in the IVDK from the 1 September 2007 to 31 December 2009 are presented in Tables 2 and 3.

Of the study population (patients tested with the series 'further fragrances') 11.07% ($n=207$) and 8.15% ($n=152$) reacted positively to FM I (after exclusion of SSO positives) and to FM II. The frequencies of sensitization in the study population (Table 4, col. 2) were extrapolated from the frequency of reactions to the single compounds (Tables 2 and 3). For example, 207 reacted positive

Table 1. Descriptive analysis of demographic and clinical factors according to the MOAHLFA index of the population tested with the series 'further fragrances'

		<i>n</i>	%
Men	M	518	26.8
Occupational	O	527	27.2
Atop. dermatitis	A	588	30.4
Hand dermatitis	H	733	37.9
Leg dermatitis	L	118	6.1
Face dermatitis	F	482	24.9
Age ≥ 40	A	1328	68.7

Table 2. Results of breakdown testing in 806 patients with positive reactions to FM I and without reactions to SSO. Results of reading at D3 (or D4)

Substance	Conc. %	%pos
Fragrance Mix I (FM I)	8.00	100
Oak moss absolue (<i>Evermia prunastri</i>)	1.00	27.4
Isoeugenol	1.00	18.2
Hydroxycitronellal	1.00	9.6
Cinnamal	1.00	9.4
Cinnamyl alcohol	1.00	8.2
Eugenol	1.00	6.7
Geraniol	1.00	3.8
Amylcinnamal	1.00	1.9
Vehicle in all preparations: petrolatum		

Table 3. Results of breakdown testing in 324 patients positive to FM II. Results of reading at D3 (or D4)

Substance	Conc. (%)	%pos
Fragrance Mix II (FM II)	14.00	100
HICC ^a	5.00	44.8
Citral	2.00	13
Farnesol	5.00	12
Hexyl cinnamal	10.00	6.2
Cumarin	5.00	4
Citronellol	1.00	2.8
Vehicle in all preparations: petrolatum.		
^a Hydroxyisohexyl 3-cyclohexene carboxaldehyde (e.g. Lyril ®)		

to FM I, of which 6.7% (table 2) would have reacted to Eugenol, which results in a frequency of sensitization of 0.74% in the study population ($n = 1870$) (Table 4, col 2).

Results of testing the compounds of the series 'further fragrances' are also presented in Table 4, col 2, marked with an asterisk.

The share of positive reactions to single compounds is presented in Table 4, col. 3. It ranges from tree moss (share of 23.6%), HICC (15.1%), oak moss (12.5%) and isoeugenol (8.3%), over hydroxycitronellal (4.4%), citral (4.4%), cinnamal (4.3%), farnesol (4%), cinnamic alcohol (3.7%), eugenol (3.0%), butylphenyl methylpropional (Lilial ®) (2.9%) and hexyl cinnamal (2.1%) down to a group of lower impact on the total of allergic reactions: geraniol (1.7%), coumarin, linalool, amylcinnamyl alcohol (each 1.3%), amyl cinnamal, benzyl salicylate, citronellol (each 0.9%), limonene, methyl 2-octynoate (methyl heptene carbonate), benzyl cinnamate, benzyl alcohol (each 0.7%), alpha-isomethyl ionone (0.4%) and anise alcohol (0.3%). No reactions to benzyl benzoate were observed in the study period.

Amount of exposure

There were two main sources to estimate the exposure: (a) the total volume sold (IFRA data) (Table 4) and (b) the occurrence on the label of products. For the latter, a larger dataset from the CVUA with 5451 products, of which 3541 leave on products were considered separately (Table 5), and from 3 smaller datasets, with 516 300 and 88 products (presented together in Table 6), were available.

Fragrances highly used in terms of the relative volume sold (standardized market share) were butylphenyl methylpropional (Lilial ®; 19 %) hexyl cinnamal (17%), linalool (16%), limonene (10%), citronellol (8%), benzyl salicylate (7%) and geraniol (6%).

Fragrances with a market share between 1% and 4% were coumarin (4%), benzyl benzoate (2%), HICC, citral, alpha-isomethyl ionone, eugenol and amyl cinnamal (each 2%). Eleven fragrances were more rarely used (<1%) (including the fragrances of FM I isoeugenol, cinnamal, cinnamic alcohol and hydroxycitronellal). Seven were very rarely (<0.1%) used: benzyl cinnamate, anise alcohol, tree moss, farnesol, oakmoss, methyl 2-octynoate (methyl heptene carbonate), in decreasing order. Amylcinnamyl alcohol was virtually not used (Table 4 col 5; Table 6 col 4 and col 6).

Regarding the frequency of occurrence on the labels the ranking of the share of labelled fragrances corresponds largely to the ranking of the use volume (market share) (Table 5 compared to Table 4) with linalool (14%), limonene (13%), citronellol (9%), geraniol (8%), butylphenyl methylpropional (Lilial ®; 7%) and hexyl cinnamal (6%) on the top of the labelling list. (For exceptions see below.) There is a good concordance of the rarely labelled fragrances with the fragrances rarely used: Isoeugenol, cinnamal, benzyl cinnamate, tree moss, oak moss, anise alcohol, amylcinnamyl alcohol and methyl 2-octynoate (methyl heptene carbonate), all below < 1%. Spearman's rank correlation coefficient between volume-based IFRA data and exposure data based on labelling from CVUA was 0.94 ($P < 0.0001$). The distribution is shown in Figure 1.

A certain difference emerges in the case of farnesol, which ranked, with 0.01%, on the last but one place (25th/26) in the volume list, which however appeared on the labels in 1.23% (18th/26). Farnesol was found to be much more often used in deodorants than in different creams^[19] [CVUA statistics: 16% (out of 129 deodorants) vs. 4% (out of 1634 creams); in the list of Rastogi et al. ($n = 88$) (Table 6, col 6), farnesol was contained in 15% (13/88).^[15] The difference between (lower) volumes and (relatively higher) labelling figures may be as a result of a relatively low concentration of farnesol in products. Furthermore, the very rare use of volume as well the (corresponding) low labelling frequency of tree moss, oak moss, anise alcohol, methyl 2-octynoate (methyl heptene carbonate) and amylcinnamyl alcohol are noteworthy.

The ranking of occurrence in the three smaller datasets (Table 6) corresponds again largely with the CVUA labelling, with limonene, linalool, butylphenyl methylpropional (Lilial ®), geraniol, alpha-isomethyl ionone, hexyl cinnamal, citronellol and benzyl salicylate at the top and with cinnamal, benzyl cinnamate, amylcinnamyl alcohol, methyl heptene carbonate, oak moss and tree moss (each < 1%) at the bottom of the three lists. However, in deodorants, oak moss (*Evernia prunastri* extract) was found to be present in 5.4% out of 129 deodorants (CVUA data), whereas in different creams it was very rarely present (0.5% out of 1634), in accordance with a study by Uter et al.^[19] Anise alcohol was very rare ($n = 1 / 0.06\%$) in the Buckley list (Table 6, col. 4), and again, in two of the datasets (Table 6), amylcinnamyl alcohol did not occur at all.

In summary, a clear and robust picture of exposure frequencies emerges from five independent sources, identifying unanimously fragrances with great, medium and low exposure from the surrogate exposure perspectives of (i) global use volume and (ii) labelling.

Sensitization Exposure Quotient (SEQ)

The SEQ per substance was calculated as the quotient of the share of allergic reactions (Table 4, col 3) divided by the share of the

Table 4. Frequencies of sensitization in $n = 1870$ tested (col. 2), and share of allergic reactions (%) (col 3), accompanied by the 95% CI (col 4). The share of volumes sold as provided by IFRA for the year 2008 ('market share') (col 5) and SEQ (col 6), relating the share of allergic reactions and the share of volume sold, as described, sorted in decreasing order. Substances contained in the series 'Further fragrances' are marked with an asterisk. The remainder are constituents of FM I and FM II

	pos %	share of positives		share of vol	SEQ
		%	CI		
Oak moss absolue (<i>Evernia prunastri</i>)	3.03	12.5	[9.6 – 15.9]	0.01	1250
Tree moss* (<i>Evernia furfuracea</i>)	5.72	23.6	[19.8 – 27.8]	0.02	1180
Farnesol	0.98	4	[2.4 – 6.3]	0.01	400
Methyl 2-octynoate (Methyl heptene carbonate)*	0.16	0.7	[0.1 – 1.9]	0.01	70
Isoeugenol	2.01	8.3	[5.9 – 11.2]	0.24	34.58
Benzyl cinnamate*	0.16	0.7	[0.1 – 1.9]	0.05	14
Cinnamal	1.04	4.3	[2.6 – 6.6]	0.32	13.44
Anise alcohol*	0.05	0.3	[0.0 – 1.2]	0.03	10
HICC ^a	3.65	15.1	[11.9 – 18.7]	1.91	7.91
Cinnamyl alcohol	0.9	3.7	[2.2 – 5.9]	0.49	7.55
Hydroxycitronellal	1.06	4.4	[2.7 – 6.7]	0.86	5.12
Citral	1.06	4.4	[2.7 – 6.7]	1.79	2.46
Eugenol	0.74	3	[1.7 – 5.1]	1.51	1.99
Benzyl alcohol*	0.16	0.7	[0.1 – 1.9]	0.9	0.78
Amyl cinnamal	0.21	0.9	[0.2 – 2.2]	1.48	0.61
Cumarin	0.33	1.3	[0.5 – 2.9]	3.53	0.37
Geraniol	0.42	1.7	[0.7 – 3.4]	6.06	0.28
Alpha-Isomethyl ionone (gamma-Methylionone)*	0.11	0.4	[0.1 – 1.6]	1.75	0.23
Butylphenyl methylpropional (Lilial ®) *	0.7	2.9	[1.5 – 4.9]	19.42	0.15
Benzyl salicylate*	0.21	0.9	[0.2 – 2.2]	7.39	0.12
Hexyl cinnamal	0.5	2.1	[1.0 – 3.9]	16.89	0.12
Citronellol	0.23	0.9	[0.3 – 2.3]	7.65	0.12
Linalool*	0.32	1.3	[0.5 – 2.9]	15.49	0.08
D,L-Limonene*	0.16	0.7	[0.1 – 1.9]	10.11	0.07
Benzyl benzoate*	0	0	[0.0 – 0.7]	2.1	0
Amylcinnamyl alcohol	0.32	1.3	[0.5 – 2.9]	0	n.c.

^aHICC: Hydroxyisohexyl 3-cyclohexene carboxaldehyde (e.g. Lyrall ®).

n.c., not calculated.

exposure (as indicated by volume or labelling) (Table 4 col 6, Table 5, col 4 and col 7, Table 7). As volumes were provided by IFRA for the year 2008, the SEQs were calculated based on sensitization rates for the study period and separately for the year 2008 only. As the results from the two periods did not differ, only the SEQs for the total study period are presented (Table 4, col 6).

The SEQ (IFRA based, Table 4, col 6) as an estimate of sensitization risk ranged from 1250 (oak moss) to limonene (0.07) and benzybenzoate (0.0). The absolute figures are meaningless, and can only be used for ranking the relative risk associated with the compounds. The SEQs of oak moss (1250), tree moss (1180), farnesol (400), methyl 2-octynoate (methyl heptene carbonate; 70) and isoeugenol (35) places them at the top of the list indicating a (very) high risk of sensitization, whereas butylphenyl methylpropional (Lilial ®; 0.15), hexyl cinnamal (0.12), citronellol (0.12), linalool (0.08), limonene (0.07) rank at the bottom, indicating a (very) low risk of sensitization.

The SEQs based on labelling frequencies are presented in Tables 5 and 7. As before, they were calculated as a quotient of the share of allergic reactions (Table 4, col 3) divided by the share of the exposure (as indicated by labelling) (Table 5 col 4 and Table 7). In general, the risk as far as identified by the SEQ (labelling) corresponds roughly with the rank of importance the single '26 EU' compounds were attributed to.^[6,8] Only amylcinnamyl

alcohol (SEQ 14.4) ranking fourth may be regarded as an outlier, probably owing to non-representative exposure (labelling) data. In fact, according to three other data sources [IFRA, Buckley and Rastogi (Tables 4 and 6)] amylcinnamyl alcohol did not figure among substances with a notable exposure. Furthermore, the 'positive' cases are put into perspective by a problematic patch test reaction profile (RI -0.1 and PR 86%) with a high number of irritant/doubtful and weak (+) positive, and thus possibly some false-positive reactions.^[6] With less true allergic reactions the SEQ would decrease.

Comparisons of SEQ using different exposure measures

Comparing the labelling and volume-based exposure approaches, there was, in general, a good correspondence of the ranking of substances (see also Figure 1), in particular substances with a great [oak moss, tree moss, methyl 2-octynoate (methyl heptene carbonate), cinnamal and isoeugenol], and a lower risk were equally identified (benzyl salicylate, benzyl alcohol, citronellol, linalool and limonene). Likewise most of the substances associated with a medium risk were equally identified. Some differences can be noted all the same (Table 8): farnesol, benzyl cinnamate, benzyl alcohol and alpha-isomethyl ionone ranked 5 to 7 places higher in the volume list than in the labelling list. One explanation could be that the frequency of labelling does not fully reflect the volume used

Table 5. Sensitization exposure quotient (SEQ) (col 4 and 7) calculated on the basis of INCI labelling frequencies from the CVUA data set for all products ($n = 5451$) and for leave-on products only ($n = 3541$) and sorted in decreasing order for all products. SEQ calculation as described, relating the share of allergic reactions (Table 4 col 3) and the share of labelling frequencies (col 3 and 6). The number of occurrences on products col 2 and 5. The slightly lower SEQs for tree moss, oak moss and others in leave-on products is due to a relative higher share (col 6 vs. col 3) in this product category

	All products			Leave on only		
	INCI labelling <i>n</i>	Share (%)	SEQ CVUA	INCI labelling <i>n</i>	Share (%)	SEQ CVUA
Tree moss (<i>Evernia furfuracea</i>)	50	0.32	73.16	33	0.34	68.43
Oak moss absolue (<i>Evernia prunastri</i>)	44	0.28	44.03	36	0.38	33.22
Methyl 2-octynoate (Methyl heptine carbonate)*	7	0.05	15.5	5	0.05	13.4
Amylcinnamyl alcohol	14	0.09	14.39	11	0.11	11.31
Cinnamal	67	0.43	9.95	45	0.47	9.14
Isoeugenol	132	0.85	9.75	97	1.01	8.19
HICC ^a	606	3.91	3.86	388	4.06	3.72
Farnesol	191	1.23	3.25	142	1.48	2.7
Anise alcohol	15	0.1	3.1	10	0.1	2.87
Cinnamyl alcohol	233	1.5	2.46	153	1.6	2.31
Benzyl cinnamate	59	0.38	1.84	39	0.41	1.72
Hydroxycitronellal	458	2.95	1.49	299	3.13	1.41
Eugenol	486	3.14	0.96	309	3.23	0.93
Citral	755	4.87	0.9	532	5.56	0.79
Amyl cinnamal	199	1.28	0.7	100	1.05	0.86
Butylphenyl methylpropional (Lilial ®) *	1018	6.57	0.44	578	6.04	0.48
Hexyl cinnamal	867	5.59	0.38	492	5.14	0.41
Cumarin	711	4.59	0.28	498	5.2	0.25
Geraniol	1271	8.2	0.21	813	8.5	0.2
Benzyl salicylate	780	5.03	0.18	507	5.3	0.17
Benzyl alcohol	605	3.9	0.18	351	3.67	0.19
Citronellol	1348	8.7	0.1	823	8.6	0.1
Linalool	2123	13.7	0.09	1207	12.61	0.1
Alpha-Isomethyl ionone (gamma-Methylionone)*	805	5.19	0.08	505	5.28	0.08
D,L-Limonene	2048	13.21	0.05	1167	12.2	0.06
Benzyl benzoate	608	3.92	0	428	4.47	0
occurrences	15500			9568		

^aHydroxyisohexyl 3-cyclohexene carboxaldehyde (e.g. Lylal ®).

probably because of a relatively low use concentration per product leading to a relative increase in labelled products and thus a lower SEQ.

In summary, the risk may be overestimated for certain compounds when referring to the frequencies of sensitization (see Table 4, col. 2) only. From a public health perspective this would not be dramatic. However, *vice versa*, the risk may heavily be underestimated which would pose a problem if not adequately considered.

Discussion

Up until now, the importance of fragrances as contact allergens is expressed only by the frequencies of sensitization found in patch test populations.^[6,7,20–23] There is a good agreement regarding the frequency of sensitization between the results of studies patch testing the '26 EU' substances,^[6,20,21,23] although in a Danish study,^[21] there were five compounds with no positive reaction [limonene, geraniol, alpha-isomethyl ionone, methyl 2-octynoate (methyl heptine carbonate) and benzyl benzoate], as in the Netherlands^[20] (amyl cinnamal, anise alcohol, benzyl cinnamate,

limonene and benzyl benzoate). By contrast, in the most recent study from St. John's, London, still sustaining the hitherto found general ranking, cinnamyl alcohol emerged as the leading allergen, ahead of oak moss, tree moss, isoeugenol and cinnamal. This may be an indicator of an increased exposure to cinnamic compounds in the very last years^[23] (see also Geier *et al.*, this issue^[18]).

Based on the results of this study (Table 4, col 2), on a former study from the IVDK^[6] and on the most recent study from St. John's,^[23] and mainly in concordance with the study from Denmark^[21] and the Netherlands,^[20] a group of frequent sensitizers is discernible (St. John's $\geq 1\%$, IVDK upper CI ≥ 1), consisting (in decreasing order) of tree moss, HICC, oak moss, isoeugenol, hydroxycitronellal, citral, cinnamal, farnesol and cinnamyl alcohol (the new impact of cinnamic compounds, cinnamyl alcohol and cinnamal, identified in the St. John's study, has been mentioned above). A second group with (very) rare sensitizers ($\leq 0.3\%$ and upper CI ≤ 0.5) consists of amyl cinnamal, benzyl salicylate, methyl 2-octynoate (methyl heptine carbonate), benzyl cinnamate, benzyl alcohol, limonene, alpha-isomethyl ionone, anise alcohol and benzyl benzoate. The remainder (with slightly different rankings in the IVDK and St. John's statistics)

Table 6. Share of INCI labelling frequencies from smaller surveys^[13–15] (col 3, 5 and 7) and sorted in decreasing order of the RIVM^a results (col 3). Frequencies of occurrence col 2, 4 and 6. Deviating results in the two other data sets in bold

# products	RIVM ^[13]		Buckley ^[14]		Rastogi et al ^[15]	
	516		300		88	
	share (%)		share (%)		share (%)	
d,l-Limonene	10	13.85	189	10.61	47	8.36
Linalool	249	10.26	190	10.67	47	8.36
Butylphenyl methylpropional (Lilial ®) *	41	7.11	126	7.07	43	7.66
Geraniol	88	6.34	126	7.07	43	7.66
Alpha-Isomethyl ionone (gamma-Methylionone)*	112	6.22	104	5.84	41	7.3
Hexyl cinnamal	128	6.11	125	7.02	29	5.17
Citronellol	109	6.05	145	8.14	58	10.32
Benzyl salicylate	114	5.33	114	6.4	35	6.23
Coumarin	79	4.87	90	5.05	29	5.17
Eugenol	81	4.5	80	4.49	24	4.28
Benzyl alcohol	185	4.39	61	3.43	15	2.68
Benzyl benzoate	76	4.21	70	3.93	22	3.92
HICC ^b	5	3.67	88	4.94	29	5.17
Citral	56	3.33	74	4.15	23	4.09
Hydroxycitronellal	60	3.1	52	2.92	24	4.28
Amyl cinnamal	110	2.26	22	1.24	9	1.6
Anise alcohol	96	2.01	1	0.06	2	0.36
Cinnamyl alcohol	12	1.83	25	1.4	11	1.96
Farnesol	66	1.12	23	1.29	13	2.32
Isoeugenol	4	0.89	27	1.52	8	1.43
Cinnamal	16	0.72	17	0.95	1	0.17
Benzyl cinnamate	36	0.66	10	0.56	3	0.53
Amylcinnamyl alcohol	20	0.54	0	0	0	0
Methyl 2-octynoate (Methyl heptine carbonate)*	33	0.29	0	0	1	0.17
Oak moss absolute (<i>Evernia prunastri</i>)	13	0.23	13	0.73	4	0.72
Tree moss (<i>Evernia furfuracea</i>)	2	0.11	9	0.51	2	0.36
Occurrences	1801		1781		563	

^aRIVM: Rijksinstituut voor Volksgezondheid en Milieu (Institute for Public Health and the Environment, Bilthoven, the Netherlands)

^bHydroxyisohexyl 3-cyclohexene carboxaldehyde (e.g. Lyril ®).

may be qualified as less frequent but not negligible: eugenol, butylphenyl methylpropional (Lilial ®), hexyl cinnamal, geraniol, coumarin, amylcinnamyl alcohol, linalool and citronellol. The cut-off between groups II and III is certainly arbitrary.

These different frequencies of sensitization could simply reflect the frequencies of use: those of group I would then be frequently used and those of group II and III more rarely. We wondered whether this explanation holds true, when referring to the actual use of fragrances. A perfunctory look on the statistics of use (in terms of volume and labelling) already shows that this is not always the case. Tree moss, for example, is the most important allergen in terms of allergy frequency, but rarely used in cosmetics (Table 5, col 3). Limonene, at the lower end of the allergy statistics, is abundantly used (Table 5, col 3).^[16] One will conclude that the risk to become sensitized to tree moss must be higher than to become sensitized to limonene.

In other substances, there may be some correlation between sensitization and use, for example in the case of citral (frequent sensitization/ frequent use) or benzyl cinnamate (infrequent sensitization/ limited use). In these cases no conclusion regarding the risk of sensitization is possible. In order to be able to compare these different allergy-use relationships, we calculated a SEQ

taking into account the relative use of substances considered for analysis and the relative frequency of sensitization. The SEQ can be regarded as an estimate of sensitization risk associated with exposure to the respective fragrance.

The risk to become sensitized is almost negligible in the cases of linalool, limonene, alpha-isomethyl ionone and benzyl benzoate, with SEQs < 0.1, remarkably according to both exposure scenarios (volume and labelling; Tables 4 and 5) and corresponding with very high EC3 values in the LLNA indicating a very low potency.^[13] However, concerning limonene as well as linalool, it must be taken into account that patch tests were performed with material which was not oxidized. Patch test preparations and ROATs with oxidized limonene or linalool were shown to cause much more positive reactions.^[24–30] The source of sensitization to the oxidized fragrances, however, is still to be elucidated, e.g. by chemical analysis^[31,32] or by patch or repeated open application testing of the suspected products.

Referring to both exposure scenarios (volume and labelling) the highest risk to be sensitized is associated with exposure to oak moss, tree moss, methyl 2-octynoate (methyl heptine carbonate), cinnamal and isoeugenol. Interestingly, these compounds were shown to be (very) potent sensitizers in the LLNA:^[7] methyl

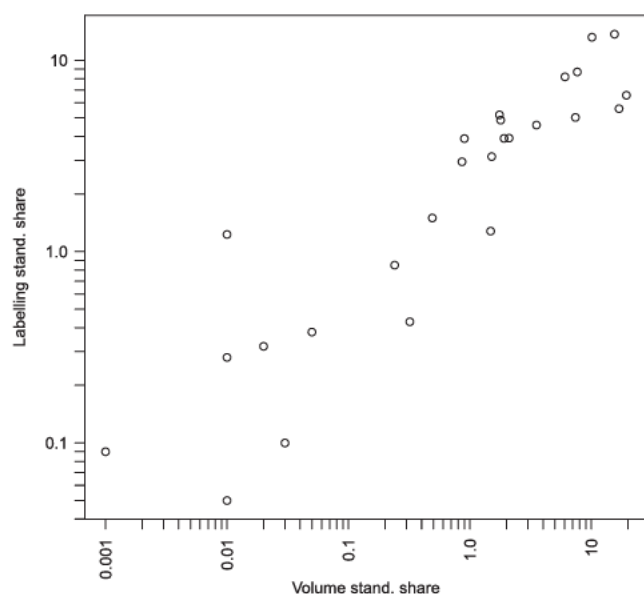


Figure 1. Correlation between volume based the International Fragrance association (IFRA) data and exposure data based on labelling from CVUA according to data shown in Tables 4 and 5. Spearman's rank correlation coefficient was 0.94 ($P < 0.0001$)

2-octynoate (methyl heptene carbonate) (<0.5% EC3), cinnamal (0.2%), isoeugenol (0.54%). In accordance with the high potency of methyl 2-octynoate is the observation of active sensitization according to a Danish study.^[21] The lichen extracts were found to have EC3 values largely > 1%,^[33] but they contain the strong sensitizers atranol (EC3 of 0.6%) and chloroatranol (EC3 of 0.4%) to various degrees.^[34] In all above cases, there is a good correlation between hazard (LLNA) and risk (SEQ).

However, risk can generally not be deduced directly from hazard, as the risk is further influenced by exposure conditions. This can be illustrated by two examples (farnesol and HICC). The sensitization prevalence to farnesol is relatively high, in spite of a low exposure [extremely low in the volume statistics (table 4), as also found in a recent study,^[16] hence the surprisingly high place in the SEQ ranking]. The high risk cannot be explained by the sensitizing potency of farnesol (EC3 > 4%), but rather by exposure conditions. Farnesol is often used in deodorants (15% and 16% of products vs. 4% in leave-on products, see above), and the axillae can be regarded as a *locus minoris resistentiae* facilitating sensitization. HICC had been the second most frequent sensitizer,^[6] (Table 4 col 2), and the SEQ ranked 7th and 9th in both exposure scenarios although endowed with only a moderate potency (EC3:17.1%).^[7] The high sensitization frequency and a relatively high risk are very probably due to (very) high use concentrations (>1% in the past,

Table 7. Sensitization exposure quotient (SEQ) calculated on the basis of INCI labelling frequencies from smaller surveys^[13–15] and sorted in decreasing order of the RIVM^a results. Deviating results in the two other data sets in bold. SEQ calculation as described, relating the share of allergic reactions (table 4 col 3) and the share of labelling frequencies (table 6)

	RIVM ^[13]	Buckley ^[14]	Rastogi et al ^[15]
Tree moss (<i>Evernia furfuracea</i>)	214.55	46.27	65.56
Oak moss absolute (<i>Evernia prunastri</i>)	54.35	17.12	17.36
Isoeugenol	9.33	5.46	5.8
Cinnamal	5.97	4.53	25.29
HICC ^b	4.11	3.06	2.92
Farnesol	3.57	3.1	1.72
Methyl 2-octynoate (Methyl heptene carbonate)*	2.41	0	4.12
Amylcinnamyl alcohol	2.41	0	0
Cinnamyl alcohol	2.02	2.64	1.89
Hydroxycitronellal	1.42	1.51	1.03
Citral	1.32	1.06	1.08
Benzyl cinnamate	1.06	1.25	1.32
Eugenol	0.67	0.67	0.7
Butylphenyl methylpropional (Lilial ®) *	0.41	0.41	0.38
Amyl cinnamal	0.4	0.73	0.56
Hexyl cinnamal	0.34	0.3	0.41
Coumarin	0.27	0.26	0.25
Geraniol	0.27	0.24	0.22
Benzyl salicylate	0.17	0.14	0.14
Benzyl alcohol	0.16	0.2	0.26
Anise alcohol	0.15	5	0.83
Citronellol	0.15	0.11	0.09
Linalool	0.13	0.12	0.16
Alpha-Isomethyl ionone (gamma-Methylionone)*	0.06	0.07	0.05
d,l-Limonene	0.05	0.07	0.08
Benzyl benzoate	0	0	0

^aRIVM: Rijksinstituut voor Volksgezondheid en Milieu (Institute for Public Health and the Environment, Bilthoven, the Netherlands)

^bHICC: Hydroxyisohexyl 3-cyclohexene carboxaldehyde (e.g. Lyrall ®)

Table 8. Comparison of Sensitization exposure quotient (SEQs) based on exposure according to volume data from IFRA vs. exposure data according to labelling from CVUA. The higher SEQ in the volume scenario [the International Fragrance association (IFRA)] of e.g. farnesol by five points is due to relatively lower exposure expressed by volume compared with labelling frequency

INCI vs. IFRA	SEQ CVUA	SEQ IFRA	rank difference
Tree moss (<i>Evernia furfuracea</i>)	73.16	1180	
Oak moss absolue (<i>Evernia prunastri</i>)	44.03	1250	
Methyl 2-octynoate (Methyl heptine carbonate)*	15.5	400	+5
Amylcinnamyl alcohol	14.39	70	-1
Cinnamal	9.95	34.58	+1
Isoeugenol	9.75	14	+5
HICC ^a	3.86	13.44	-2
Farnesol	3.25	10	+1
Anise alcohol	3.1	7.91	-2
Cinnamyl alcohol	2.46	7.55	
Benzyl cinnamate	1.84	5.12	+1
Hydroxycitronellal	1.49	2.46	+2
Eugenol	0.96	1.99	
Citral	0.9	0.78	+7
Amyl cinnamal	0.7	0.61	
Butylphenyl methylpropional (Lilial ®)*	0.44	0.37	+2
Hexyl cinnamal	0.38	0.28	+2
Coumarin	0.28	0.23	+6
Geraniol	0.21	0.15	-3
Benzyl salicylate	0.18	0.12	
Benzyl alcohol	0.18	0.12	-4
Citronellol	0.1	0.12	
Linalool	0.09	0.08	
Alpha-Isomethyl ionone (gamma-Methylionone)*	0.08	0.07	+1
D,L-Limonene	0.05	0	
Benzyl benzoate	0	n.c.	
Amylcinnamyl alcohol			

^aHICC: Hydroxyisohexyl 3-cyclohexene carboxaldehyde (e.g. Lyrall ®).

and up to 0.2% in 2007) and also frequent use in deodorants. The recommended use concentration is 0.02% for those product categories considered to pose the greatest risk of sensitization since 2009.^[7,35,36]

Although the frequency of sensitization statistics was found to reflect the risk as indicated by the SEQs in some frequent (oak moss, tree moss and isoeugenol) and some rare allergens (<1% share of positives): namely amyl cinnamal, benzyl salicylate, citronellol, benzyl alcohol, limonene, alpha-isomethyl ionone, there are also divergences between the two rankings meaning that the frequency of sensitization may not fully reflect the associated risk.

The risk SEQ ranked lower than indicated by the frequency of sensitization in the case of HICC (5 points lower), hydroxycitronellal (7), citral (8), butylphenyl methylpropional (Lilial ®; 5), hexyl cinnamal (5), geraniol (6) and linalool (7). The risk ranked much higher than the ranking according to frequency of sensitization in the case of amylcinnamyl alcohol (10 points higher), benzylcinnamate (10), anise alcohol (16) and methyl 2-octynoate (methyl heptine carbonate) (17). Amylcinnamyl alcohol (SEQ 14.4) ranked fourth may be regarded as an outlier, probably owing to non-representative exposure (labelling) data. In fact, according to three other data sources (IFRA, Buckley and Rastogi) amylcinnamyl alcohol did not figure among substances with a

countable exposure. Sensitization is probably caused by exposure to other cinnamic compounds.

This means: the risk may be overestimated for certain compounds when referring to the frequencies of sensitization only. From a public health perspective this would not be dramatic. However, the risk may heavily be underestimated which would pose a problem if not adequately considered. In these cases exposure conditions (e.g. use concentrations) should be re-evaluated.

Limitations

This first attempt to relate allergy frequencies to fragrances and their use in cosmetics has limitations to be considered:

1. Selection of products may not be sufficiently representative, despite the considerable number of products (>5000). However, there is mostly good concordance when ranking exposure between the different scenarios (volume versus labelling). More recent surveys support the ranking presented in this study.^[16]
2. Not all but the major manufacturers are members of IFRA. According to IFRA, the production and sold volumes of non-members are about 10 to 20%. However, if the volumes not

- reported were included in the calculation, the SEQ would further decrease, thus the resulting SEQs are conservative estimates.
- It cannot be excluded that if in a few high volume 'blockbuster' products (e.g. from large discounters) or in high volume non-cosmetic products (e.g. fabric wash) the fragrances contained deviate from the overall pattern the representation of exposure to these fragrances would be distorted – namely, underestimated for those fragrances which are more commonly found in the high volume products, resulting in a higher SEQ. The SEQ would then reflect a 'worst case' situation.
 - Some fragrances are used in non-cosmetic areas such as detergents,^[16] and some sensitization may be acquired through these sources. However, detergents play a very limited role in sensitization.^[37] Mainly, limonene, linalool, hexyl cinnamal, butylphenyl methylpropional (Lilial®) and citronellol are used.^[16] Other non-cosmetic exposure (e.g. aromatherapy, pharmaceuticals, occupational materials) may also contribute to the overall exposure, albeit presumably to a limited extent. Including such exposures would have increased the share of products and reduced the SEQ of these substances further.
 - Establishing a realistic exposure estimate would require an aggregate exposure assessment. Aggregate exposure is defined as the total exposure to one substance that arises from multiple sources via different pathways and routes. Moreover, mixture exposure (to several substances at the same time) seems, according to recent experimental findings, to increase the risk of sensitization and elicitation to the fragrances involved.^[38] A recent study examined the pattern of co-exposure to fragrances in different categories of cosmetics from the German market.^[19] According to the CVUA statistics referred to in this study a product contained 2.8 fragrance compounds on average.
 - A more precise indicator of exposure would have been the quantity applied per area of the skin. Such data are not available on larger scales.

Conclusion

By relating the relative frequencies of sensitization to the relative frequencies of use in products serving as a marker of exposure, we were able to show that the risk to be sensitized is – as expected – only partially reflected by the absolute number of sensitization, particularly in the case of oak moss, tree moss, isoeugenol and cinnamal on the one hand and limonene, alpha-isomethyl ionone, citronellol and benzyl alcohol on the other. In other cases, the crude figures would overestimate the risk and can be put into perspective by a high exposure, namely in the case of butylphenyl methylpropional (Lilial®), hexyl cinnamal, geraniol and linalool. In principle, this reasoning could also be applied to HICC, hydroxycitronellal and citral, but regardless of the relatively low calculated risk associated with these compounds, high sensitization frequencies have been observed and must be considered as a warning signal in their own right, prompting preventive measures.

In contrast, those cases are more important where the risk ranked much higher than the ranking according to frequency of sensitization, namely in benzyl cinnamate, anise alcohol and methyl 2-octynoate (methyl heptene carbonate). Low sensitization rates may mislead fragrance manufacturers to using the compounds more than would be beneficial.

Although there is no point-to-point correlation between risk and hazard (as expressed by sensitizing potency), substances found to

pose a low risk were classified as weak to non-sensitizers, and substances with a high risk were classified as moderate to strong sensitizers in predictive animal testing thus corroborating our approach of relative risk assessment. Vice versa, the hazard assessed by predictive testing is obviously the most important (primary) determinant of risk, which may be compensated only secondarily by adequately adjusted concentrations of exposure. The reverse is true as well: HICC was found to be a sensitizer of only moderate to weak potency (EC3 value 17.1%/0.81 M).^[7,39] The SEQ ranks HICC at the seventh (or ninth) place (out of 26), indicating a high, but at least not an extreme risk. However, sensitization very probably following too high use concentrations over longer periods was so widespread that the SCCS recommended the substance not to be used in cosmetics any longer.^[7]

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Conflicts of interest

The IVDK is sponsored by the cosmetic and fragrance industry (associations). W.U. has accepted honoraria for presentations or travel reimbursement from cosmetic industry associations. A.S. works as ad-hoc consultant for cosmetic industry (associations), partly remunerated. The other authors have no conflicts to declare.

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DELINEATION OF COVALENT BOND FORMATION-DEPENDENT FROM REACTIVE OXYGEN SPECIES-MEDIATED INDUCTION OF LUCIFERASE EXPRESSION IN KERATINOSENS™ ASSAY

Introduction

Keratinosens™ is one of the *in vitro* assays that test the impact of chemical compounds on defined key points of the adverse outcome pathway (1, 2). The basis of the test is as follows. Keratinocyte cell line transfected with luciferase under control of ARE (Anti-oxidant Response Element) enhancer found in human AKR1C2 (Aldo-Keto Reductase Family 1, member C2) gene is treated with test chemicals. If chemicals are able to form covalent bonds with Cysteine in Keap1 (Kelch like ECH-Associated Protein 1), the reaction will induce dissociation of Keap1 from Nrf2 (Nuclear Factor like 2) in the cytoplasm. As a consequence of dissociation, Nrf2 will translocate to the nucleus, bind to the ARE enhancer and induce luciferase expression. Thus, Keratinosens™ is thought to test reactivity of chemicals to peptides in cellular surrounding.

However, direct evidence for Keap1 modification is missing. Furthermore, reactive chemicals can induce reactive oxygen species (ROS)(3), which in turn can affect Keap1-Nrf2 association (4-7). Therefore, the mechanism of luciferase induction by test chemicals may be mediated via various types of mechanisms, including covalent bond formation (key event 1 of the adverse outcome pathway) and ROS induction (that may lead to key event 2). There are a number of chemicals that test positive in *in chemico* DPRA1 test, but not in Keratinosens™ assay, and vice versa (2). While some of these discrepancies may theoretically be ascribed to different conditions of *in chemico* and *in vitro* assays, the alternative explanation may be that Keratinosens™ assay may score positive even if the test chemical does not directly bind to the Keap1 Cys.

The purpose of this project is to address the cellular mechanism(s) of luciferase induction in Keratinosens™. We will investigate whether ROS induction occurs following introduction of fragrances and whether ROS production is responsible for luciferase expression. This may lead to further experiments aimed at direct evaluation of chemical(s) binding to Keap1 (e.g. mass spectrometry analysis of Keap1 after treatment with chemical). Overall, the proposed experiments should help delineate mechanisms by which different fragrances contribute to hypersensitivity reactions.

Specific aims

- 1) Luciferase expression induction by chemicals in the absence or presence of ROS quenchers (SOD mimetics). This should tell us if the luciferase induction by chemicals is mediated by ROS.
- 2) Induction of ROS by chemicals with different outcome profiles in DPRA1 and KeratinosensTM. In the absence or presence of SOD mimetics. This should tell us if chemicals induce ROS.
Conceptually and logically, this should be done first, but some of the data is already known and we'd like to get to the point (the mechanism of luciferase expression) sooner rather than later.

Experimental design

Aim 1. Luciferase expression induction by chemicals in the absence or presence of ROS quenchers (SOD mimetics).

Choice of ROS quenchers. MnTBAP chloride (CAS 55266-18-7) is a cell permeable superoxide dismutase mimetic and peroxynitrite scavenger (8-10).

Choice of test chemicals.

Chemical	Rationale	ROS induction
Iodoacetamide	Forms covalent bonds with Cys without inducing ROS	No
Paraquat	Superoxide ion generator (9)	Yes
Pyocyanine	Superoxide ion generator (11)	Yes
Hydrogen peroxide	Hydrogen peroxide (8)	Yes
Peroxynitrite	Peroxynitrite (12)	Yes
R(+) Limonene	Positive in DPRA1, negative in Keratinosens TM (2)	
d,l-Citronellol	Positive in DPRA1, negative in Keratinosens TM (2)	
Lillial	Positive in DPRA1, negative in Keratinosens TM (2)	Yes (3)
Coumarin	Negative in DPRA1, positive in Keratinosens TM (2)	
Geraniol	Negative in DPRA1, positive in Keratinosens TM (2)	
Benzyl Cinnamate	Negative in DPRA1, positive in Keratinosens TM (2)	
Benzyl benzoate	Negative in DPRA1, positive in Keratinosens TM (2)	
Farnesol	Negative in DPRA1, positive in Keratinosens TM (2)	
Benzyl salicylate	Negative in DPRA1, positive in Keratinosens TM (2)	

Hexylcinnamaldehyde	Negative in DPRA1, borderline in Keratinosens™ (2)	Not above 2x threshold (3)
p-Phenyldiamine	Positive in both DPRA1 and Keratinosens™ (2)	No (3)
Isoeugenol	Positive in both DPRA1 and Keratinosens™ (2)	Yes (3)
Cinnamic aldehyde	Positive in both DPRA1 and Keratinosens™ (2)	Yes (3)
Citral	Positive in both DPRA1 and Keratinosens™ (2)	Yes (3)

Experiment. Pick a concentration known to induce positive results in DPRA1 and/or Keratinosens™ (or take a range of working concentrations) and test luciferase induction in the absence or presence of a range of MnTBAP concentrations.

Aim 2. Induction of ROS by chemicals with different score patterns in DPRA1 and Keratinosens™. In the absence or presence of SOD mimetics.

Choice of fluorescent ROS probes. Hydroxiethidine (HE) is used for detection of reactive oxygen radicals while dichlorodihydrofluorescein (DCFH) detects hydrogen peroxide. Is there a need for a general ROS indicator, e.i. dichlorodihydrofluorescein diacetate?

Choice of chemicals. Same as in Aim 1.

Experiment. Preload cells with probes and then treat with chemicals. Short time thereafter (e.g. 5 min) analyze by flow cytometry.

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From: [Vukmanovic, Stanislav](#)
To: [Sadrieh, Nakissa](#); [Ikhlas Khan](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#); [Herrmann, Michelle](#)
Subject: RE: keratinosens
Date: Thursday, December 10, 2015 11:45:14 AM
Attachments: [Keratinosens project v1.docx](#)

Dear Ikhlas and Amar,

In order to have a more productive discussion tomorrow, it may be a good idea to share with you the preliminary plan of the project that we would like to be performed. That way the discussion can be more informative. Your input in design is welcome.

Best,

Stan

From: Sadrieh, Nakissa
Sent: Thursday, December 10, 2015 11:01 AM
To: Ikhlas Khan
Cc: Vukmanovic, Stanislav; AMAR GOPAL CHITTIBOYINA
Subject: Re: keratinosens

Tomorrow is better for me. Maybe we can talk sometime after 2 pm? I will check with my calendar and confirm. Thanks.

From: Ikhlas Khan <ikhlan@olemiss.edu>
Date: December 10, 2015 at 10:31:28 AM EST
To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Cc: Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: Re: keratinosens
Importance: High

Dear Nakissa

I am back in office and had discussion with Amar and others. Please let me know when you have time for discussion. We are available this afternoon and also tomorrow. If it does not work for you, please suggest for next week

Thanks

IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, December 2, 2015 at 8:20 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>

Subject: Re: keratinosens

Thank you Ikhlas. Once I hear from you, we can discuss our proposed study.

From: Ikhlas Khan <ikhlan@olemiss.edu>

Date: December 2, 2015 at 9:18:38 PM EST

To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>

Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>

Subject: Re: keratinosens

Dear Nakissa

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To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>

Subject: keratinosens

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Regards,
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Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: [Vukmanovic, Stanislav](#)
To: [Ikhlas Khan](#); [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#); [Herrmann, Michelle](#); [SHABANA I KHAN](#)
Subject: RE: keratinosens
Date: Wednesday, February 17, 2016 2:29:31 PM
Attachments: [26f in vitro project v2.docx](#)

Thank you so much for your update. I can imagine that there may be a number of hurdles one has to jump over to get this license. Patience is a virtue...

While we are waiting, there is another project that would like to initiate. Part of this project, too, will require Keratinosens license, but the other parts should be doable. In a nutshell, we would like to start generating independent in vitro data on allergens using validated methods (DPRA, Keratinosens, and h-CLAT). We would like to start with the 26 fragrances (hence the attached proposal talks only about these compounds), but the project would eventually expand to other potential allergens. Could you please have a look at the attached proposal and let us know your thoughts.

Thanks again for all your efforts.

Best,

Stan

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Wednesday, February 17, 2016 1:55 PM
To: Vukmanovic, Stanislav; Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle; SHABANA I KHAN
Subject: Re: keratinosens

Dear Stan

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From: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Date: Monday, January 25, 2016 at 12:30 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>, shabana <skhan@olemiss.edu>
Subject: RE: keratinosens

Great! Thank you so much.

Best,

Stan

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Monday, January 25, 2016 1:26 PM
To: Vukmanovic, Stanislav; Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle; SHABANA I KHAN
Subject: Re: keratinosens

Hi Stan

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we will keep you posted

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Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>
Subject: RE: keratinosens

Hi Ikhlas,

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I just wanted to touch base and see whether by now you may have an idea how easy (or difficult) will it be for you to acquire the license for Keratinocyte™ assay.

Please, let us know any updates you may have.

Best,

Stan

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Friday, December 11, 2015 10:56 AM
To: Sadrieh, Nakissa; Vukmanovic, Stanislav
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle
Subject: Re: keratinosens

Dear Nakissa

We can call you at your office# or you can give a number to call. I assume it's 2:00 CST it's 2 EST your time I will request to delay it to 3:EST or 2:30 EST
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From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

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To: Ikhlas Khan <ikhlan@olemiss.edu>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>

Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>

Subject: RE: keratinosens

Ikhlas, are we still talking at 2 pm today? What telephone number would you like us to call, or are you calling us? Thanks.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
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Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Thursday, December 10, 2015 12:00 PM

To: Vukmanovic, Stanislav; Sadrieh, Nakissa

Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle

Subject: Re: keratinosens

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ik

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Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>

Subject: RE: keratinosens

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Cc: [AMAR GOPAL CHITTIBOYINA](#); [Herrmann, Michelle](#); [SHABANA I KHAN](#)
Subject: Re: keratinosens
Date: Wednesday, April 06, 2016 5:05:59 PM

Dear Nakissa and Stan

After making several rounds of conference call with different departments at University and company in Switzerland, its clear that company won't change their policy and University can't accept their conditions.

Nut after talking to our Lawyer at the university, he suggested some other mechanism which probably will work. It means we can have cell lines in near future.
I will keep you posted.

IK

P.S: Nakissa, I assume you all set for the trip, here is my cell# (b) (6)

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4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

Tsai, Victoria

From: Sadrieh, Nakissa
Sent: Thursday, June 15, 2017 2:37 PM
To: Moghaddam, Sarvin
Subject: FW: Manuscript on 24 fragrance ingredients
Attachments: dcya_fragrances draft 05302017.docx

Follow Up Flag: Follow up
Flag Status: Flagged

Categories: Red Category

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
5001 Campus Drive
Room 1042 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Amar Chittiboyina [mailto:amar@olemiss.edu]
Sent: Wednesday, May 31, 2017 5:48 PM
To: Sadrieh, Nakissa; Vukmanovic, Stanislav
Cc: Ikhlas A. Khan; Cristina Avonto
Subject: Manuscript on 24 fragrance ingredients

Dear Nakissa and Stan,
Draft on stability and reactivity of 24 fragrance ingredients using in-chemico (DCYA) method is attached. Please review the manuscript and let us know when we can submit it to toxicology journals and your input is very valuable. At this point, we are thinking to send it to Toxicology In vitro. If you see any other journal as a better fit, let us know.
Thanks a lot,
Amar

Amar Chittiboyina, PhD
Assistant Director, NCNPR
311H TCRC West, School of Pharmacy, University, MS 38655
[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)
pharmacy.olemiss.edu/ncnpr/

Tsai, Victoria

From: Amar Chittiboyina <amar@olemiss.edu>
Sent: Wednesday, April 19, 2017 9:22 AM
To: Moghaddam, Sarvin
Subject: FW: Meeting today
Attachments: hCLAT final results_24compounds_April2017.xlsx; DCYA report of 24 fragrances 04112017.docx

FYI.

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)
pharmacy.olemiss.edu/ncnpr/

From: Amar Chittiboyina [mailto:amar@olemiss.edu]
Sent: Thursday, April 13, 2017 5:08 PM
To: 'Moghaddam, Sarvin'; 'Ikhlas Khan'; 'CRISTINA AVONTO'; 'Shabana Khan'
Cc: 'Vukmanovic, Stanislav'; 'Sadrieh, Nakissa'
Subject: RE: Meeting today

Hello Sarvin,

As per your request, the data on hCLAT and HTS-DCYA is attached. The validation of KeratinoSens is completed in our labs and we are in process of testing these 24 ingredients.

It's the time again for the conference call with your team and suggest us your convenient time.

Thank you and have a good weekend

Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

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Sent: Friday, March 31, 2017 12:33 PM
To: Amar Chittiboyina; 'Ikhlas Khan'; 'CRISTINA AVONTO'; Shabana Khan
Cc: Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Hello Amar,

Thank you for the update on the project status. We have some questions and requests for the further directions of our joint projects:

- 1) Following our last meeting, our understanding was that you have hCLAT and HTS-DCYA results as well as Cys-DPRA and Lys-DPRA results for the 24 fragrance allergens. However, in the "Progress Report_Set1_24 Ingredients.doc" report provided on March 9th, only Cys-DPRA and Lys-DPRA results for the 24 fragrance allergens are provided. We would like to see if you can update the report with hCLAT and HTS-DCYA results and also KeratinoSens results at later time.
- 2) Following some discussion in our group, we would like to expand the project with some new ingredients. Attached, please find the list of ingredients including the initial 24 fragrance allergens for which available results have already been populated.
- 3) Finally, a while ago when we were talking about introducing KeratinoSens assay we discussed a project on delineating the covalent bond-mediated effects from those mediated by reactive oxygen radicals. To refresh our memories, project description is attached. Since KeratinoSens appears to be finally in place, we would like to ask you to make a plan (and send it to us) on carrying this project forward.

Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Friday, March 03, 2017 3:45 PM
To: Sadrieh, Nakissa; 'Ikhlas Khan'
Cc: 'CRISTINA AVONTO'; Moghaddam, Sarvin; Vukmanovic, Stanislav; Shabana Khan
Subject: RE: Meeting today

Hello Nakissa,

Thank you for the call and it is helpful for us to identify the priorities and goals. As you suggested, we will schedule the teleconference calls for every two months to abreast our research efforts.

In addition to the DPRA report on 24 ingredients, the protocols we are implementing at NCNPR is also attached. Let us know if you need any additional information.

Thanks,
Amar

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Sent: Friday, February 17, 2017 10:27 AM
To: 'Ikhlas Khan'
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Moghaddam, Sarvin; Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Do you have a telephone number that we can call? We can try to find a conference room, and then call you. Sarvin and Stan will be joining the discussion. if you want to call us, our conference room telephone number is (b) (6) We will likely use that line. thank you.

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)

4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Friday, February 17, 2017 10:05 AM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: Meeting today

Sure, lets do at 4:00 to be safe.
Which number should we call
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, February 17, 2017 at 7:07 AM
To: ikhlas <ikhlan@olemiss.edu>
Subject: Meeting today

Ikhlas,

We have had a meeting scheduled at 2 pm on the other campus, and I won't be back till 3:30. The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

Tsai, Victoria

From: Sadrieh, Nakissa
Sent: Thursday, March 09, 2017 12:13 PM
To: 'Ikhlas Khan'; AMAR GOPAL CHITTIBOYINA
Cc: Moghaddam, Sarvin; Vukmanovic, Stanislav
Subject: FW: Meeting today
Attachments: Set2_24 Compounds.xlsx; Common plant allergens_02202017.xlsx; Protocol_DPRA.DOCX; Protocol_hCLAT.DOCX; Protocol HTS-DCYA.DOCX; Protocol_NMR-DCYA_.docx; Progress Report_Set1_24 Ingredients.docx

Hi Ikhlas,

I just wanted to respond to the email that you just me, and to let you know that we have receive Amar's email, with the attachments. we will get back to you shortly, so that we can get started with the studies.

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Amar Chittiboyina [mailto:amar@olemiss.edu]
Sent: Friday, March 03, 2017 3:45 PM
To: Sadrieh, Nakissa; 'Ikhlas Khan'
Cc: 'CRISTINA AVONTO'; Moghaddam, Sarvin; Vukmanovic, Stanislav; Shabana Khan
Subject: RE: Meeting today

Hello Nakissa,

Thank you for the call and it is helpful for us to identify the priorities and goals. As you suggested, we will schedule the teleconference calls for every two months to abreast our research efforts.

In addition to the DPRA report on 24 ingredients, the protocols we are implementing at NCNPR is also attached. Let us know if you need any additional information.

Thanks,
Amar

From: Sadrieh, Nakissa [<mailto:Nakissa.Sadrieh@fda.hhs.gov>]

Sent: Friday, February 17, 2017 10:27 AM

To: 'Ikhlas Khan'

Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Moghaddam, Sarvin; Vukmanovic, Stanislav; Sadrieh, Nakissa

Subject: RE: Meeting today

Do you have a telephone number that we can call? We can try to find a conference room, and then call you. Sarvin and Stan will be joining the discussion. if you want to call us, our conference room telephone number is (b) (6) We will likely use that line. thank you.

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]

Sent: Friday, February 17, 2017 10:05 AM

To: Sadrieh, Nakissa

Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO

Subject: Re: Meeting today

Sure, lets do at 4:00 to be safe.

Which number should we call

ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Friday, February 17, 2017 at 7:07 AM

To: ikhlas <ikhan@olemiss.edu>

Subject: Meeting today

Ikhlas,

We have had a meeting scheduled at 2 pm on the other campus, and I won't be back till 3:30. The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

Tsai, Victoria

From: Sadrieh, Nakissa
Sent: Tuesday, January 24, 2017 7:18 PM
To: Moghaddam, Sarvin
Subject: Fwd: NCNPR-CFSAN monthly meeting
Attachments: Collaborative research efforts with CFSAN.DOCX; Cosmetic Work_NCNPR 012017.pdf

Follow Up Flag: Follow up
Flag Status: Flagged

Categories: Red Category

Hello,

I wanted to give you some material from our research projects at the University of Mississippi. We might be talking with them next Friday (February 3), via phone.

From: Amar Chittiboyina <amar@olemiss.edu>
Date: January 24, 2017 at 6:22:16 PM EST
To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>, 'Ikhlas Khan' <ikhan@olemiss.edu>
Subject: RE: NCNPR-CFSAN monthly meeting

The materials to familiarize your team are attached and let us know if you need any additional information. Thanks, -
Amar

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Tuesday, January 24, 2017 4:52 PM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA
Subject: Re: NCNPR-CFSAN monthly meeting

Sounds good, IS next Friday Feb 3rd morning will work for?
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Tuesday, January 24, 2017 at 4:06 PM
To: ikhlas <ikhan@olemiss.edu>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: RE: NCNPR-CFSAN monthly meeting

Hello,

I think that next week would be good for us to talk. Can you please send me a status of the various projects and assays that you have available for cosmetics research? I have new staff that I wish to assign to working with you, and I want to familiarize them with the previous work, as well as the ongoing work and what we have discussed as planned future projects. So next week would be a good time to talk. Thank you.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Tuesday, January 24, 2017 4:01 PM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA
Subject: Re: NCNPR-CFSAN monthly meeting

Hi Nakissa

I know you are busy but I think we should talk about the future work whenever you have time
IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Tuesday, January 10, 2017 at 10:48 AM
To: ikhlas <ikhlan@olemiss.edu>, Cara Welch <Cara.Welch@fda.hhs.gov>, "Swift, Sibyl" <Sibyl.Swift@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Eric Olson <Eric.Olson@fda.hhs.gov>
Cc: JT <jnnfrtyl@olemiss.edu>
Subject: RE: NCNPR-CFSAN monthly meeting

I think that we can postpone the call to another time, since we are preparing for the apocalypse here (January 20th). but I look forward to a discussion after that. we have ideas for jump starting our research for 2017.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Tuesday, January 10, 2017 11:06 AM
To: Welch, Cara; Swift, Sibyl; Sadrieh, Nakissa; AMAR GOPAL CHITTIBOYINA; Olson, Eric
Cc: Jennifer S. Taylor
Subject: Re: NCNPR-CFSAN monthly meeting

Nakissa, let us know if you want to me on or we can call some other time
ik

From: Cara Welch <Cara.Welch@fda.hhs.gov>
Date: Tuesday, January 10, 2017 at 7:39 AM
To: "Swift, Sibyl" <Sibyl.Swift@fda.hhs.gov>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Eric Olson <Eric.Olson@fda.hhs.gov>, ikhlas <ikhlan@olemiss.edu>
Cc: JT <jnnfrtyl@olemiss.edu>
Subject: RE: NCNPR-CFSAN monthly meeting

ODSP needs to cancel this month's meeting, Thursday, Jan 12 – OCAC or NCNPR, do you want to keep the meeting amongst yourselves?
Cara

-----Original Appointment-----

From: Welch, Cara
Sent: Thursday, October 13, 2016 3:27 PM
To: Welch, Cara; Swift, Sibyl; Sadrieh, Nakissa; Amar Chittiboyina (amar@olemiss.edu); Olson, Eric; ikhlan@olemiss.edu
Cc: Jennifer S. Taylor
Subject: NCNPR-CFSAN monthly meeting
When: Thursday, January 12, 2017 2:00 PM-3:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: phone

-- Do not delete or change any of the following text. --
You are the host for this Personal Conference meeting.

Meeting Number: (b) (6)

To start the audio portion of the Personal Conference meeting

1. Please call one of the following numbers:

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2. Follow what you hear on the phone.

Cisco Unified MeetingPlace meeting ID: (b) (6)

Cisco Unified MeetingPlace profile number for meeting host (b) (6)

FDARichMedia@fda.hhs.gov

IMPORTANT NOTICE: This WebEx service includes a feature that allows audio and any documents and other materials exchanged or viewed during the session to be recorded. You should inform all meeting attendees prior to recording if you intend to record the meeting. Please note that any such recordings may be subject to discovery in the event of litigation.

Tsai, Victoria

From: Moghaddam, Sarvin
Sent: Thursday, August 03, 2017 4:24 PM
To: Amar Chittiboyina (amar@olemiss.edu)
Cc: Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: products with Aloe ingredient
Attachments: Aloe - products - GNPD-download - 08032017.rtf; Aloe- products - GNPD-download-2017-08-03_20_21_58.xls

Hi Amar,

Attached please find the products with Aloe ingredients. Please let me know if you need any additional information. Both files represent the same products in different formats.

Thank you
Sarvin

Tsai, Victoria

From: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Sent: Wednesday, July 26, 2017 11:34 AM
To: Moghaddam, Sarvin
Subject: Re: Meeting next week?

I am working on it, will send the invitation to you all shortly. Thanks, -Amar

From: Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov>
Sent: Wednesday, July 26, 2017 9:30:54 AM
To: AMAR GOPAL CHITTIBOYINA
Subject: RE: Meeting next week?

Or I can add you all to the invitation and send it again. Only, please provide me with the number to call in.

Thanks
Sarvin

From: Moghaddam, Sarvin
Sent: Wednesday, July 26, 2017 10:30 AM
To: 'AMAR GOPAL CHITTIBOYINA'
Subject: RE: Meeting next week?

Hi Amar,
Sorry I missed this email of yours and noticed it today. Do you mind to setup the meeting as I have already sent my invitation and some of us might work from home that day so it is better if we call you.

Thanks
Sarvin

From: AMAR GOPAL CHITTIBOYINA [mailto:amar@olemiss.edu]
Sent: Monday, July 24, 2017 5:45 PM
To: Moghaddam, Sarvin
Subject: Re: Meeting next week?

Hi Sarvin,
Great! Can you setup the meeting with number to call in? If you want me, I can do it from my end. Thanks, -Amar
When you send the invitation, please include the following three individuals too.

1. Ikhlas Khan - ikhlan@olemiss.edu
2. Shabana Khan - skhan@olemiss.edu
3. Cristina Avonto- cavonto@olemiss.edu

Thanks a lot and looking forward to chat with you
Amar

AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>

From: Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov>
Sent: Monday, July 24, 2017 2:21:17 PM
To: AMAR GOPAL CHITTIBOYINA
Subject: FW: Meeting next week?

Hi Amar,
Both Nakissa and Stand accepted the meeting invitation. We are all set for the meeting.

Thanks,
Sarvin

From: Moghaddam, Sarvin
Sent: Monday, July 24, 2017 10:53 AM
To: 'AMAR GOPAL CHITTIBOYINA'
Subject: RE: Meeting next week?

Hi Amar,
I sent the meeting invitation to both Nakissa and Stan and as soon as they accept the meeting, I will let you know.

Thank you
Sarvin

From: AMAR GOPAL CHITTIBOYINA [<mailto:amar@olemiss.edu>]
Sent: Friday, July 21, 2017 6:43 PM
To: Moghaddam, Sarvin
Subject: Re: Meeting next week?

I checked with Dr. Khan and others in our group and 31st July at 1:00pm CST is preferred. Once you confirm, I will notify our folks.

Thanks,
Amar

From: Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov>
Sent: Friday, July 21, 2017 1:25:43 PM
To: AMAR GOPAL CHITTIBOYINA
Subject: RE: Meeting next week?

Hi Amar,
Below please find the times that work for Nakissa.

July 31 afternoon
August 2nd after 2:30pm
August 3rd afternoon

Thanks

Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Thursday, July 20, 2017 2:34 PM
To: Moghaddam, Sarvin
Subject: RE: Meeting next week?

Going well at our end and hope everything is ok at your end. Why don't we start from Nakissa's available dates and timing for next week? Give me 2-3 options. Thanks, Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)
pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Thursday, July 20, 2017 12:48 PM
To: Amar Chittiboyina
Subject: Meeting next week?

Hi Amar

I hope all is well.

I was wondering if you can let me know of your group available times to schedule an update meeting for next week so I can see which one works with our schedule. Specially, Nakissa has a very tight schedule as I can see in her calendar.

Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Monday, June 19, 2017 11:01 AM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

Sure will. Looking forward to talk to you. Thanks, -A

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)
pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Monday, June 19, 2017 9:58 AM
To: Amar Chittiboyina
Subject: RE: Meeting today

Hi Amar,

I don't think I can schedule any meeting this week that fits everybody's schedule and next week I am in training all week. I will be in touch early in July. In case, you have any additional data by then, please let us know, if not, we will be in touch for our meeting.

Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Friday, June 16, 2017 2:22 PM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

Hello Sarvin,

We do have some additional results, however, would like to re-confirm before sending you. So, do not wait for additional data, go ahead and let us know time and day for phone call.

Thanks
Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Thursday, June 15, 2017 2:24 PM
To: Amar Chittiboyina
Subject: RE: Meeting today

Hi Amar,
Hope all is well.

We like to setup an update meeting and was wondering if you have any new results you may like to share before the meeting.

Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Friday, May 05, 2017 2:36 PM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

Hello Sarvin,

As you requested, along with data produced at NCNPR with alternative methods (DCYA, DPRA, KeratinoSens and hCLAT), the literature data is also included. The evaluation with KeratinoSens is ongoing and will pass onto you, once it is ready. Let us know if you need any additional information and looking forward for the conference call. Have a good weekend.
Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]

Sent: Wednesday, May 03, 2017 1:54 PM

To: Amar Chittiboyina

Subject: RE: Meeting today

Anytime tomorrow will work for me.

thanks

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]

Sent: Wednesday, May 03, 2017 2:53 PM

To: Moghaddam, Sarvin

Subject: RE: Meeting today

I am already tied up until 2 CST. Suggest me some other time or day. Thanks, Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

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pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]

Sent: Wednesday, May 03, 2017 11:36 AM

To: AMAR GOPAL CHITTIBOYINA

Subject: RE: Meeting today

I just noticed you have 2 central time. I might have a meeting at 3 o'clock our time.

So Maybe earlier?

From: AMAR GOPAL CHITTIBOYINA [<mailto:amar@olemiss.edu>]

Sent: Wednesday, May 03, 2017 12:32 PM

To: Moghaddam, Sarvin

Subject: Re: Meeting today

I will call you for a quick chat. So that both of us on same page. May be around 2:00 pm CST.

Amar

Sent from Amar's iPhone

On May 3, 2017, at 9:51 AM, Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov> wrote:

Hi Amar,

No worries at all and thanks for getting back to me. We all have our busy days!

I understand your concern about % difference, what do you think about just having the reference “positive” or “negative” classification. Please let me know what you think or please feel free to call me if you think it is easier.

Thank you
Sarvin
(240)402-1154

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Monday, May 01, 2017 10:17 AM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

Hello Sarvin,
My apologies. Last week, I was tied up with time-sensitive, employee development issues. Certainly can be done. What do mean by % difference between NCNPR and literature results? If you meant number to number for each compound, I don't think it is good idea. Because, the assays we are using are sensitive and the number(s) depended on purity of the compound. And, most of the compounds purity depends on sample age, how they are stored etc.
Thanks,
Amar

Amar Chittiboyina, PhD
Assistant Director, NCNPR
311H TCRC West, School of Pharmacy, University, MS 38655
[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)
pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Monday, May 01, 2017 8:54 AM
To: Amar Chittiboyina (amar@olemiss.edu)
Subject: FW: Meeting today

Hi Amar,

Hope all is well,
I appreciate if you let me know what you think of my email below?

Thank you
Sarvin

From: Moghaddam, Sarvin
Sent: Tuesday, April 25, 2017 11:12 AM
To: 'Amar Chittiboyina'
Subject: RE: Meeting today

Hi Amar,

We were wondering if you can add extra columns to the existing columns (in the attached file) and include the literature results that you are evaluating your in-house results against and also the % difference between your results and the available literature results.

Thank you,
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Thursday, April 20, 2017 2:54 PM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

Refer hCLAT data in mmc.xlsx file.

Amar Chittiboyina, PhD
Assistant Director, NCNPR
311H TCRC West, School of Pharmacy, University, MS 38655
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pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Thursday, April 20, 2017 1:52 PM
To: Amar Chittiboyina
Subject: RE: Meeting today

Can you send me the Excel file if you have it handy?

Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Thursday, April 20, 2017 2:51 PM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

Please check the Urbisch 2015 supplementary data in Excel format. Let me know if you need any additional information. Thanks, Amar

Amar Chittiboyina, PhD
Assistant Director, NCNPR
311H TCRC West, School of Pharmacy, University, MS 38655
[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)
pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Thursday, April 20, 2017 1:01 PM
To: AMAR GOPAL CHITTIBOYINA
Subject: RE: Meeting today

Just for clarification, you are not comparing all your results with Nukada paper? Since, I only see 11 out of 24 ingredients in Nukada paper. Please correct me if I am wrong?
As for the asterisks, I see 4 ingredients and two are reported in Nukada paper.
Attached please find your file, I only have highlighted the ingredients that are included in Nukada paper.

I will check the other paper shortly.

Thank you
Sarvin

From: AMAR GOPAL CHITTIBOYINA [<mailto:amar@olemiss.edu>]
Sent: Thursday, April 20, 2017 1:34 PM
To: Moghaddam, Sarvin
Subject: Re: Meeting today

Disagreement data is indicated with asterisk. Only 5 compounds marked and I believe they are in Nukada's reported data.

Sent from Amar's iPhone

On Apr 20, 2017, at 11:31 AM, Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov> wrote:

Hi Amar,
Reviewing the data provided in "Copy of hCLAT final results_24compounds_April2017 (3).xlsx" file, you have in subtitle:

P: Positive; N: negative; * The result classification is based on two out of three in-house experiments, however

However, Nukada paper doesn't provide data for all the 24 ingredients. What "reported literature classification" for h-CLAT results do you refer to?

Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Wednesday, April 19, 2017 9:22 AM
To: Moghaddam, Sarvin
Subject: FW: Meeting today

FYI.

Amar Chittiboyina, PhD
Assistant Director, NCNPR
311H TCRC West, School of Pharmacy, University, MS 38655
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pharmacy.olemiss.edu/ncnpr/

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Thursday, April 13, 2017 5:08 PM
To: 'Moghaddam, Sarvin'; 'Ikhlas Khan'; 'CRISTINA AVONTO'; 'Shabana Khan'
Cc: 'Vukmanovic, Stanislav'; 'Sadrieh, Nakissa'
Subject: RE: Meeting today

Hello Sarvin,

As per your request, the data on hCLAT and HTS-DCYA is attached. The validation of KeratinoSens is completed in our labs and we are in process of testing these 24 ingredients.

It's the time again for the conference call with your team and suggest us your convenient time.

Thank you and have a good weekend
Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Friday, March 31, 2017 12:33 PM
To: Amar Chittiboyina; 'Ikhlas Khan'; 'CRISTINA AVONTO'; Shabana Khan
Cc: Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Hello Amar,

Thank you for the update on the project status. We have some questions and requests for the further directions of our joint projects:

- 1) Following our last meeting, our understanding was that you have hCLAT and HTS-DCYA results as well as Cys-DPRA and Lys-DPRA results for the 24 fragrance allergens. However, in the "Progress Report_Set1_24 Ingredients.doc" report provided on March 9th, only Cys-DPRA and Lys-DPRA results for the 24 fragrance allergens are provided. We would like to see if you can update the report with hCLAT and HTS-DCYA results and also KeratinoSens results at later time.
- 2) Following some discussion in our group, we would like to expand the project with some new ingredients. Attached, please find the list of ingredients including the initial 24 fragrance allergens for which available results have already been populated.
- 3) Finally, a while ago when we were talking about introducing KeratinoSens assay we discussed a project on delineating the covalent bond-mediated effects from those mediated by reactive oxygen radicals. To refresh our memories, project description is attached. Since KeratinoSens appears to be finally in place, we would like to ask you to make a plan (and send it to us) on carrying this project forward.

Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Friday, March 03, 2017 3:45 PM
To: Sadrieh, Nakissa; 'Ikhlas Khan'
Cc: 'CRISTINA AVONTO'; Moghaddam, Sarvin; Vukmanovic, Stanislav; Shabana Khan
Subject: RE: Meeting today

Hello Nakissa,
Thank you for the call and it is helpful for us to identify the priorities and goals. As you suggested, we will schedule the teleconference calls for every two months to abreast our research efforts.

In addition to the DPRA report on 24 ingredients, the protocols we are implementing at NCNPR is also attached. Let us know if you need any additional information.

Thanks,
Amar

From: Sadrieh, Nakissa [<mailto:Nakissa.Sadrieh@fda.hhs.gov>]
Sent: Friday, February 17, 2017 10:27 AM
To: 'Ikhlas Khan'
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Moghaddam, Sarvin; Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Do you have a telephone number that we can call? We can try to find a conference room, and then call you. Sarvin and Stan will be joining the discussion. if you want to call us, our conference room telephone number is (b) (6) We will likely use that line. thank you.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Friday, February 17, 2017 10:05 AM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: Meeting today

Sure, lets do at 4:00 to be safe.
Which number should we call
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, February 17, 2017 at 7:07 AM
To: ikhlas <ikhlan@olemiss.edu>
Subject: Meeting today

Ikhlas,

We have had a meeting scheduled at 2 pm on the other campus, and I won't be back till 3:30. The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

Tsai, Victoria

From: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Sent: Monday, July 31, 2017 10:30 AM
To: Moghaddam, Sarvin
Subject: RE: Meeting next week?
Attachments: Alternative Methods_Binary Classification_Set1_07312017.xlsx

Sarvin,
Please use the attached Excel file with KeratinoSens data.

From: Moghaddam, Sarvin [mailto:Sarvin.Moghaddam@fda.hhs.gov]
Sent: Wednesday, July 26, 2017 10:48 AM
To: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: RE: Meeting next week?

I received it - thanks

From: AMAR GOPAL CHITTIBOYINA [mailto:amar@olemiss.edu]
Sent: Wednesday, July 26, 2017 11:34 AM
To: Moghaddam, Sarvin
Subject: Re: Meeting next week?

I am working on it, will send the invitation to you all shortly. Thanks, -Amar

From: Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov>
Sent: Wednesday, July 26, 2017 9:30:54 AM
To: AMAR GOPAL CHITTIBOYINA
Subject: RE: Meeting next week?

Or I can add you all to the invitation and send it again. Only, please provide me with the number to call in.

Thanks
Sarvin

From: Moghaddam, Sarvin
Sent: Wednesday, July 26, 2017 10:30 AM
To: 'AMAR GOPAL CHITTIBOYINA'
Subject: RE: Meeting next week?

Hi Amar,
Sorry I missed this email of yours and noticed it today. Do you mind to setup the meeting as I have already sent my invitation and some of us might work from home that day so it is better if we call you.

Thanks
Sarvin

From: AMAR GOPAL CHITTIBOYINA [mailto:amar@olemiss.edu]
Sent: Monday, July 24, 2017 5:45 PM

To: Moghaddam, Sarvin
Subject: Re: Meeting next week?

Hi Sarvin,

Great! Can you setup the meeting with number to call in? If you want me, I can do it from my end. Thanks, -
Amar

When you send the invitation, please include the following three individuals too.

1. Ikhlas Khan - ikhlan@olemiss.edu
2. Shabana Khan - skhan@olemiss.edu
3. Cristina Avonto- cavonto@olemiss.edu

Thanks a lot and looking forward to chat with you
Amar

AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>

From: Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov>
Sent: Monday, July 24, 2017 2:21:17 PM
To: AMAR GOPAL CHITTIBOYINA
Subject: FW: Meeting next week?

Hi Amar,
Both Nakissa and Stand accepted the meeting invitation. We are all set for the meeting.

Thanks,
Sarvin

From: Moghaddam, Sarvin
Sent: Monday, July 24, 2017 10:53 AM
To: 'AMAR GOPAL CHITTIBOYINA'
Subject: RE: Meeting next week?

Hi Amar,
I sent the meeting invitation to both Nakissa and Stan and as soon as they accept the meeting, I will let you know.

Thank you
Sarvin

From: AMAR GOPAL CHITTIBOYINA [<mailto:amar@olemiss.edu>]
Sent: Friday, July 21, 2017 6:43 PM
To: Moghaddam, Sarvin
Subject: Re: Meeting next week?

I checked with Dr. Khan and others in our group and 31st July at 1:00pm CST is preferred. Once you confirm, I will notify our folks.

Thanks,
Amar

From: Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov>
Sent: Friday, July 21, 2017 1:25:43 PM
To: AMAR GOPAL CHITTIBOYINA
Subject: RE: Meeting next week?

Hi Amar,
Below please find the times that work for Nakissa.

July 31 afternoon
August 2nd after 2:30pm
August 3rd afternoon

Thanks
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Thursday, July 20, 2017 2:34 PM
To: Moghaddam, Sarvin
Subject: RE: Meeting next week?

Going well at our end and hope everything is ok at your end. Why don't we start from Nakissa's available dates and timing for next week? Give me 2-3 options. Thanks, Amar

Amar Chittiboyina, PhD
Assistant Director, NCNPR
311H TCRC West, School of Pharmacy, University, MS 38655
[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)
pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Thursday, July 20, 2017 12:48 PM
To: Amar Chittiboyina
Subject: Meeting next week?

Hi Amar
I hope all is well.
I was wondering if you can let me know of your group available times to schedule an update meeting for next week so I can see which one works with our schedule. Specially, Nakissa has a very tight schedule as I can see in her calendar.

Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Monday, June 19, 2017 11:01 AM

To: Moghaddam, Sarvin
Subject: RE: Meeting today

Sure will. Looking forward to talk to you. Thanks, -A

Amar Chittiboyina, PhD

Assistant Director, NCNPR

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pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]

Sent: Monday, June 19, 2017 9:58 AM

To: Amar Chittiboyina

Subject: RE: Meeting today

Hi Amar,

I don't think I can schedule any meeting this week that fits everybody's schedule and next week I am in training all week. I will be in touch early in July. In case, you have any additional data by then, please let us know, if not, we will be in touch for our meeting.

Thank you

Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]

Sent: Friday, June 16, 2017 2:22 PM

To: Moghaddam, Sarvin

Subject: RE: Meeting today

Hello Sarvin,

We do have some additional results, however, would like to re-confirm before sending you. So, do not wait for additional data, go ahead and let us know time and day for phone call.

Thanks

Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]

Sent: Thursday, June 15, 2017 2:24 PM

To: Amar Chittiboyina

Subject: RE: Meeting today

Hi Amar,

Hope all is well.

We like to setup an update meeting and was wondering if you have any new results you may like to share before the meeting.

Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Friday, May 05, 2017 2:36 PM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

Hello Sarvin,
As you requested, along with data produced at NCNPR with alternative methods (DCYA, DPRA, KeratinoSens and hCLAT), the literature data is also included. The evaluation with KeratinoSens is ongoing and will pass onto you, once it is ready. Let us know if you need any additional information and looking forward for the conference call. Have a good weekend.
Amar

Amar Chittiboyina, PhD
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pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Wednesday, May 03, 2017 1:54 PM
To: Amar Chittiboyina
Subject: RE: Meeting today

Anytime tomorrow will work for me.
thanks

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Wednesday, May 03, 2017 2:53 PM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

I am already tied up until 2 CST. Suggest me some other time or day. Thanks, Amar

Amar Chittiboyina, PhD
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pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Wednesday, May 03, 2017 11:36 AM
To: AMAR GOPAL CHITTIBOYINA
Subject: RE: Meeting today

I just noticed you have 2 central time. I might have a meeting at 3 o'clock our time.
So Maybe earlier?

From: AMAR GOPAL CHITTIBOYINA [<mailto:amar@olemiss.edu>]
Sent: Wednesday, May 03, 2017 12:32 PM
To: Moghaddam, Sarvin
Subject: Re: Meeting today

I will call you for a quick chat. So that both of us on same page. May be around 2:00 pm CST.
Amar

Sent from Amar's iPhone

On May 3, 2017, at 9:51 AM, Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov> wrote:

Hi Amar,
No worries at all and thanks for getting back to me. We all have our busy days!
I understand your concern about % difference, what do you think about just having the reference
"positive" or "negative" classification. Please let me know what you think or please feel free to call me if
you think it is easier.

Thank you
Sarvin
(240)402-1154

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Monday, May 01, 2017 10:17 AM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

Hello Sarvin,
My apologies. Last week, I was tied up with time-sensitive, employee development issues. Certainly can
be done. What do mean by % difference between NCNPR and literature results? If you meant number to
number for each compound, I don't think it is good idea. Because, the assays we are using are sensitive
and the number(s) depended on purity of the compound. And, most of the compounds purity depends
on sample age, how they are stored etc.
Thanks,
Amar

Amar Chittiboyina, PhD
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pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Monday, May 01, 2017 8:54 AM
To: Amar Chittiboyina (amar@olemiss.edu)
Subject: FW: Meeting today

Hi Amar,

Hope all is well,
I appreciate if you let me know what you think of my email below?

Thank you
Sarvin

From: Moghaddam, Sarvin
Sent: Tuesday, April 25, 2017 11:12 AM
To: 'Amar Chittiboyina'
Subject: RE: Meeting today

Hi Amar,

We were wondering if you can add extra columns to the existing columns (in the attached file) and include the literature results that you are evaluating your in-house results against and also the % difference between your results and the available literature results.

Thank you,
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Thursday, April 20, 2017 2:54 PM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

Refer hCLAT data in mmc.xlsx file.

Amar Chittiboyina, PhD
Assistant Director, NCNPR
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pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Thursday, April 20, 2017 1:52 PM
To: Amar Chittiboyina
Subject: RE: Meeting today

Can you send me the Excel file if you have it handy?

Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Thursday, April 20, 2017 2:51 PM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

Please check the Urbisch 2015 supplementary data in Excel format. Let me know if you need any additional information. Thanks, Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)
pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]

Sent: Thursday, April 20, 2017 1:01 PM

To: AMAR GOPAL CHITTIBOYINA

Subject: RE: Meeting today

Just for clarification, you are not comparing all your results with Nukada paper? Since, I only see 11 out of 24 ingredients in Nukada paper. Please correct me if I am wrong?

As for the asterisks, I see 4 ingredients and two are reported in Nukada paper.

Attached please find your file, I only have highlighted the ingredients that are included in Nukada paper.

I will check the other paper shortly.

Thank you

Sarvin

From: AMAR GOPAL CHITTIBOYINA [<mailto:amar@olemiss.edu>]

Sent: Thursday, April 20, 2017 1:34 PM

To: Moghaddam, Sarvin

Subject: Re: Meeting today

Disagreement data is indicated with asterisk. Only 5 compounds marked and I believe they are in Nukada's reported data.

Sent from Amar's iPhone

On Apr 20, 2017, at 11:31 AM, Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov> wrote:

Hi Amar,

Reviewing the data provided in "Copy of hCLAT final results_24compounds_April2017 (3).xlsx" file, you have in subtitle:

P: Positive; N: negative; * The result classification is based on two out of three in-house experiments, however

However, Nukada paper doesn't provide data for all the 24 ingredients. What "reported literature classification" for h-CLAT results do you refer to?

Thank you

Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Wednesday, April 19, 2017 9:22 AM
To: Moghaddam, Sarvin
Subject: FW: Meeting today

FYI.

Amar Chittiboyina, PhD

Assistant Director, NCNPR
311H TCRC West, School of Pharmacy, University, MS 38655
[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)
pharmacy.olemiss.edu/ncnpr/

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Thursday, April 13, 2017 5:08 PM
To: 'Moghaddam, Sarvin'; 'Ikhlas Khan'; 'CRISTINA AVONTO'; 'Shabana Khan'
Cc: 'Vukmanovic, Stanislav'; 'Sadrieh, Nakissa'
Subject: RE: Meeting today

Hello Sarvin,

As per your request, the data on hCLAT and HTS-DCYA is attached. The validation of KeratinoSens is completed in our labs and we are in process of testing these 24 ingredients.

It's the time again for the conference call with your team and suggest us your convenient time.

Thank you and have a good weekend
Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR
311H TCRC West, School of Pharmacy, University, MS 38655
[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)
pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Friday, March 31, 2017 12:33 PM
To: Amar Chittiboyina; 'Ikhlas Khan'; 'CRISTINA AVONTO'; Shabana Khan
Cc: Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Hello Amar,

Thank you for the update on the project status. We have some questions and requests for the further directions of our joint projects:

- 1) Following our last meeting, our understanding was that you have hCLAT and HTS-DCYA results as well as Cys-DPRA and Lys-DPRA results for the 24 fragrance allergens. However, in the "Progress Report_Set1_24 Ingredients.doc" report provided on March 9th, only Cys-DPRA and Lys-

DPRA results for the 24 fragrance allergens are provided. We would like to see if you can update the report with hCLAT and HTS-DCYA results and also KeratinoSens results at later time.

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- 3) Finally, a while ago when we were talking about introducing KeratinoSens assay we discussed a project on delineating the covalent bond-mediated effects from those mediated by reactive oxygen radicals. To refresh our memories, project description is attached. Since KeratinoSens appears to be finally in place, we would like to ask you to make a plan (and send it to us) on carrying this project forward.

Thank you
Sarvin

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To: Sadrieh, Nakissa; 'Ikhlas Khan'
Cc: 'CRISTINA AVONTO'; Moghaddam, Sarvin; Vukmanovic, Stanislav; Shabana Khan
Subject: RE: Meeting today

Hello Nakissa,

Thank you for the call and it is helpful for us to identify the priorities and goals. As you suggested, we will schedule the teleconference calls for every two months to abreast our research efforts.

In addition to the DPRA report on 24 ingredients, the protocols we are implementing at NCNPR is also attached. Let us know if you need any additional information.

Thanks,
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Sent: Friday, February 17, 2017 10:27 AM
To: 'Ikhlas Khan'
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Moghaddam, Sarvin; Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Do you have a telephone number that we can call? We can try to find a conference room, and then call you. Sarvin and Stan will be joining the discussion. if you want to call us, our conference room telephone number is (b) (6) We will likely use that line. thank you.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

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Sent: Friday, February 17, 2017 10:05 AM
To: Sadrieh, Nakissa
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Subject: Re: Meeting today

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Subject: Meeting today

Ikhlas,

We have had a meeting scheduled at 2 pm on the other campus, and I won't be back till 3:30. The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

Tsai, Victoria

From: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Sent: Monday, July 31, 2017 11:39 AM
To: Moghaddam, Sarvin
Subject: RE: Meeting next week?

These 24 are part of 87 ingredients! We are studying 24 compounds at a time for ease of experimentation and convenience.

From: Moghaddam, Sarvin [mailto:Sarvin.Moghaddam@fda.hhs.gov]
Sent: Monday, July 31, 2017 10:33 AM
To: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: RE: Meeting next week?

Hi Amar,
Looking at the "talking points" in the meeting invitation email, I see #2 as:
[Ongoing research with second set of 24 compounds](#)

I believe this list with 24 ingredients was provided to you before I joined the group. I provided a list of 87 ingredients (including the original 24 ingredients + oak moss and tree moss i.e. List of Ingredients.docx) on 4/13. Although, I should say that this list includes all the ingredients in your list (Copy of Set2_24 Compounds.xlsx) provided to us on 3/31/2017.

I am just bringing up the issue just to be on the same page about the list of ingredients.

Thanks
Sarvin

From: AMAR GOPAL CHITTIBOYINA [mailto:amar@olemiss.edu]
Sent: Monday, July 31, 2017 10:30 AM
To: Moghaddam, Sarvin
Subject: RE: Meeting next week?

Sarvin,
Please use the attached Excel file with KeratinoSens data.

From: Moghaddam, Sarvin [mailto:Sarvin.Moghaddam@fda.hhs.gov]
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Subject: RE: Meeting next week?

I received it - thanks

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Sent: Wednesday, July 26, 2017 11:34 AM
To: Moghaddam, Sarvin
Subject: Re: Meeting next week?

I am working on it, will send the invitation to you all shortly. Thanks, -Amar

From: Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov>
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Or I can add you all to the invitation and send it again. Only, please provide me with the number to call in.

Thanks
Sarvin

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Hi Amar,
Sorry I missed this email of yours and noticed it today. Do you mind to setup the meeting as I have already sent my invitation and some of us might work from home that day so it is better if we call you.

Thanks
Sarvin

From: AMAR GOPAL CHITTIBOYINA [<mailto:amar@olemiss.edu>]
Sent: Monday, July 24, 2017 5:45 PM
To: Moghaddam, Sarvin
Subject: Re: Meeting next week?

Hi Sarvin,
Great! Can you setup the meeting with number to call in? If you want me, I can do it from my end. Thanks, -Amar
When you send the invitation, please include the following three individuals too.

1. Ikhlas Khan - ikhlan@olemiss.edu
2. Shabana Khan - skhan@olemiss.edu
3. Cristina Avonto- cavonto@olemiss.edu

Thanks a lot and looking forward to chat with you
Amar

AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>

From: Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov>
Sent: Monday, July 24, 2017 2:21:17 PM
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Hi Amar,
Both Nakissa and Stand accepted the meeting invitation. We are all set for the meeting.

Thanks,
Sarvin

From: Moghaddam, Sarvin
Sent: Monday, July 24, 2017 10:53 AM
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Hi Amar,
I sent the meeting invitation to both Nakissa and Stan and as soon as they accept the meeting, I will let you know.

Thank you
Sarvin

From: AMAR GOPAL CHITTIBOYINA [<mailto:amar@olemiss.edu>]
Sent: Friday, July 21, 2017 6:43 PM
To: Moghaddam, Sarvin
Subject: Re: Meeting next week?

I checked with Dr. Khan and others in our group and 31st July at 1:00pm CST is preferred. Once you confirm, I will notify our folks.

Thanks,
Amar

From: Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov>
Sent: Friday, July 21, 2017 1:25:43 PM
To: AMAR GOPAL CHITTIBOYINA
Subject: RE: Meeting next week?

Hi Amar,
Below please find the times that work for Nakissa.

July 31 afternoon
August 2nd after 2:30pm
August 3rd afternoon

Thanks
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Thursday, July 20, 2017 2:34 PM

To: Moghaddam, Sarvin
Subject: RE: Meeting next week?

Going well at our end and hope everything is ok at your end. Why don't we start from Nakissa's available dates and timing for next week? Give me 2-3 options. Thanks, Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]

Sent: Thursday, July 20, 2017 12:48 PM

To: Amar Chittiboyina

Subject: Meeting next week?

Hi Amar

I hope all is well.

I was wondering if you can let me know of your group available times to schedule an update meeting for next week so I can see which one works with our schedule. Specially, Nakissa has a very tight schedule as I can see in her calendar.

Thank you

Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]

Sent: Monday, June 19, 2017 11:01 AM

To: Moghaddam, Sarvin

Subject: RE: Meeting today

Sure will. Looking forward to talk to you. Thanks, -A

Amar Chittiboyina, PhD

Assistant Director, NCNPR

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pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]

Sent: Monday, June 19, 2017 9:58 AM

To: Amar Chittiboyina

Subject: RE: Meeting today

Hi Amar,

I don't think I can schedule any meeting this week that fits everybody's schedule and next week I am in training all week. I will be in touch early in July. In case, you have any additional data by then, please let us know, if not, we will be in touch for our meeting.

Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Friday, June 16, 2017 2:22 PM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

Hello Sarvin,

We do have some additional results, however, would like to re-confirm before sending you. So, do not wait for additional data, go ahead and let us know time and day for phone call.

Thanks
Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

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From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Thursday, June 15, 2017 2:24 PM
To: Amar Chittiboyina
Subject: RE: Meeting today

Hi Amar,

Hope all is well.

We like to setup an update meeting and was wondering if you have any new results you may like to share before the meeting.

Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Friday, May 05, 2017 2:36 PM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

Hello Sarvin,

As you requested, along with data produced at NCNPR with alternative methods (DCYA, DPRA, KeratinoSens and hCLAT), the literature data is also included. The evaluation with KeratinoSens is ongoing and will pass onto you, once it is ready. Let us know if you need any additional information and looking forward for the conference call. Have a good weekend.
Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

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pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Wednesday, May 03, 2017 1:54 PM
To: Amar Chittiboyina
Subject: RE: Meeting today

Anytime tomorrow will work for me.
thanks

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Wednesday, May 03, 2017 2:53 PM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

I am already tied up until 2 CST. Suggest me some other time or day. Thanks, Amar

Amar Chittiboyina, PhD

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From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Wednesday, May 03, 2017 11:36 AM
To: AMAR GOPAL CHITTIBOYINA
Subject: RE: Meeting today

I just noticed you have 2 central time. I might have a meeting at 3 o'clock our time.
So Maybe earlier?

From: AMAR GOPAL CHITTIBOYINA [<mailto:amar@olemiss.edu>]
Sent: Wednesday, May 03, 2017 12:32 PM
To: Moghaddam, Sarvin
Subject: Re: Meeting today

I will call you for a quick chat. So that both of us on same page. May be around 2:00 pm CST.
Amar

Sent from Amar's iPhone

On May 3, 2017, at 9:51 AM, Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov> wrote:

Hi Amar,

No worries at all and thanks for getting back to me. We all have our busy days!

I understand your concern about % difference, what do you think about just having the reference

“positive” or “negative” classification. Please let me know what you think or please feel free to call me if you think it is easier.

Thank you

Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Monday, May 01, 2017 10:17 AM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

Hello Sarvin,

My apologies. Last week, I was tied up with time-sensitive, employee development issues. Certainly can be done. What do mean by % difference between NCNPR and literature results? If you meant number to number for each compound, I don't think it is good idea. Because, the assays we are using are sensitive and the number(s) depended on purity of the compound. And, most of the compounds purity depends on sample age, how they are stored etc.

Thanks,
Amar

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From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Monday, May 01, 2017 8:54 AM
To: Amar Chittiboyina (amar@olemiss.edu)
Subject: FW: Meeting today

Hi Amar,

Hope all is well,
I appreciate if you let me know what you think of my email below?

Thank you
Sarvin

From: Moghaddam, Sarvin
Sent: Tuesday, April 25, 2017 11:12 AM
To: 'Amar Chittiboyina'
Subject: RE: Meeting today

Hi Amar,

We were wondering if you can add extra columns to the existing columns (in the attached file) and include the literature results that you are evaluating your in-house results against and also the % difference between your results and the available literature results.

Thank you,
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Thursday, April 20, 2017 2:54 PM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

Refer hCLAT data in mmc.xlsx file.

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From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Thursday, April 20, 2017 1:52 PM
To: Amar Chittiboyina
Subject: RE: Meeting today

Can you send me the Excel file if you have it handy?

Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Thursday, April 20, 2017 2:51 PM
To: Moghaddam, Sarvin
Subject: RE: Meeting today

Please check the Urbisch 2015 supplementary data in Excel format. Let me know if you need any additional information. Thanks, Amar

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Subject: RE: Meeting today

Just for clarification, you are not comparing all your results with Nukada paper? Since, I only see 11 out of 24 ingredients in Nukada paper. Please correct me if I am wrong?
As for the asterisks, I see 4 ingredients and two are reported in Nukada paper.
Attached please find your file, I only have highlighted the ingredients that are included in Nukada paper.

I will check the other paper shortly.

Thank you
Sarvin

From: AMAR GOPAL CHITTIBOYINA [<mailto:amar@olemiss.edu>]
Sent: Thursday, April 20, 2017 1:34 PM
To: Moghaddam, Sarvin
Subject: Re: Meeting today

Disagreement data is indicated with asterisk. Only 5 compounds marked and I believe they are in Nukada's reported data.

Sent from Amar's iPhone

On Apr 20, 2017, at 11:31 AM, Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov> wrote:

Hi Amar,
Reviewing the data provided in "Copy of hCLAT final results_24compounds_April2017 (3).xlsx" file, you have in subtitle:

P: Positive; N: negative; * The result classification is based on two out of three in-house experiments, however

However, Nukada paper doesn't provide data for all the 24 ingredients. What "reported literature classification" for h-CLAT results do you refer to?

Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Wednesday, April 19, 2017 9:22 AM
To: Moghaddam, Sarvin
Subject: FW: Meeting today

FYI.

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pharmacy.olemiss.edu/ncnpr/

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Thursday, April 13, 2017 5:08 PM
To: 'Moghaddam, Sarvin'; 'Ikhlas Khan'; 'CRISTINA AVONTO'; 'Shabana Khan'
Cc: 'Vukmanovic, Stanislav'; 'Sadrieh, Nakissa'
Subject: RE: Meeting today

Hello Sarvin,

As per your request, the data on hCLAT and HTS-DCYA is attached. The validation of KeratinoSens is completed in our labs and we are in process of testing these 24 ingredients.

It's the time again for the conference call with your team and suggest us your convenient time.

Thank you and have a good weekend

Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

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pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]

Sent: Friday, March 31, 2017 12:33 PM

To: Amar Chittiboyina; 'Ikhlas Khan'; 'CRISTINA AVONTO'; Shabana Khan

Cc: Vukmanovic, Stanislav; Sadrieh, Nakissa

Subject: RE: Meeting today

Hello Amar,

Thank you for the update on the project status. We have some questions and requests for the further directions of our joint projects:

- 1) Following our last meeting, our understanding was that you have hCLAT and HTS-DCYA results as well as Cys-DPRA and Lys-DPRA results for the 24 fragrance allergens. However, in the "Progress Report_Set1_24 Ingredients.doc" report provided on March 9th, only Cys-DPRA and Lys-DPRA results for the 24 fragrance allergens are provided. We would like to see if you can update the report with hCLAT and HTS-DCYA results and also KeratinoSens results at later time.
- 2) Following some discussion in our group, we would like to expand the project with some new ingredients. Attached, please find the list of ingredients including the initial 24 fragrance allergens for which available results have already been populated.
- 3) Finally, a while ago when we were talking about introducing KeratinoSens assay we discussed a project on delineating the covalent bond-mediated effects from those mediated by reactive oxygen radicals. To refresh our memories, project description is attached. Since KeratinoSens appears to be finally in place, we would like to ask you to make a plan (and send it to us) on carrying this project forward.

Thank you

Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]

Sent: Friday, March 03, 2017 3:45 PM

To: Sadrieh, Nakissa; 'Ikhlas Khan'

Cc: 'CRISTINA AVONTO'; Moghaddam, Sarvin; Vukmanovic, Stanislav; Shabana Khan
Subject: RE: Meeting today

Hello Nakissa,

Thank you for the call and it is helpful for us to identify the priorities and goals. As you suggested, we will schedule the teleconference calls for every two months to abreast our research efforts.

In addition to the DPRA report on 24 ingredients, the protocols we are implementing at NCNPR is also attached. Let us know if you need any additional information.

Thanks,
Amar

From: Sadrieh, Nakissa [<mailto:Nakissa.Sadrieh@fda.hhs.gov>]
Sent: Friday, February 17, 2017 10:27 AM
To: 'Ikhlas Khan'
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Moghaddam, Sarvin; Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Do you have a telephone number that we can call? We can try to find a conference room, and then call you. Sarvin and Stan will be joining the discussion. if you want to call us, our conference room telephone number is 2 (b) (6) We will likely use that line. thank you.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Friday, February 17, 2017 10:05 AM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: Meeting today

Sure, lets do at 4:00 to be safe.
Which number should we call
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, February 17, 2017 at 7:07 AM

To: ikhlas <ikhlan@olemiss.edu>

Subject: Meeting today

Ikhlas,

We have had a meeting scheduled at 2 pm on the other campus, and I won't be back till 3:30. The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

Tsai, Victoria

From: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Sent: Friday, July 21, 2017 6:43 PM
To: Moghaddam, Sarvin
Subject: Re: Meeting next week?

I checked with Dr. Khan and others in our group and 31st July at 1:00pm CST is preferred. Once you confirm, I will notify our folks.

Thanks,
Amar

From: Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov>
Sent: Friday, July 21, 2017 1:25:43 PM
To: AMAR GOPAL CHITTIBOYINA
Subject: RE: Meeting next week?

Hi Amar,
Below please find the times that work for Nakissa.

July 31 afternoon
August 2nd after 2:30pm
August 3rd afternoon

Thanks
Sarvin

From: Amar Chittiboyina [mailto:amar@olemiss.edu]
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Going well at our end and hope everything is ok at your end. Why don't we start from Nakissa's available dates and timing for next week? Give me 2-3 options. Thanks, Amar

Amar Chittiboyina, PhD
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Sent: Thursday, July 20, 2017 12:48 PM
To: Amar Chittiboyina
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So Maybe earlier?

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Sent from Amar's iPhone

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Hi Amar,
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I understand your concern about % difference, what do you think about just having the reference
"positive" or "negative" classification. Please let me know what you think or please feel free to call me if
you think it is easier.

Thank you
Sarvin
(240)402-1154

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number for each compound, I don't think it is good idea. Because, the assays we are using are sensitive
and the number(s) depended on purity of the compound. And, most of the compounds purity depends
on sample age, how they are stored etc.
Thanks,
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Refer hCLAT data in mmc.xlsx file.

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To: 'Moghaddam, Sarvin'; 'Ikhlas Khan'; 'CRISTINA AVONTO'; 'Shabana Khan'
Cc: 'Vukmanovic, Stanislav'; 'Sadrieh, Nakissa'
Subject: RE: Meeting today

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Subject: RE: Meeting today

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Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Moghaddam, Sarvin;
Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

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Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Friday, February 17, 2017 10:05 AM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: Meeting today

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Date: Friday, February 17, 2017 at 7:07 AM
To: ikhlas <ikhlan@olemiss.edu>
Subject: Meeting today

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Tsai, Victoria

From: Moghaddam, Sarvin
Sent: Friday, March 31, 2017 1:33 PM
To: Amar Chittiboyina; 'Ikhlas Khan'; 'CRISTINA AVONTO'; Shabana Khan
Cc: Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today
Attachments: Keratinosens project v1.docx; List of Ingredients.docx

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Sent: Friday, March 03, 2017 3:45 PM
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Cc: 'CRISTINA AVONTO'; Moghaddam, Sarvin; Vukmanovic, Stanislav; Shabana Khan
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Hello Sarvin,

Thank you for the update. We are in process of finalizing the data (HTS-DCYA, hCLAT) on 24 compounds, shall send it to you shortly. Meanwhile we will explore the possible sources and acquire the suggested compounds. Yes, we did not forget about Stan's proposal on Reactive chemicals, induction of ROS and assessment with KeratinoSens. Currently we are validating the KeratinoSens assay in our labs by testing the known compounds. Once the validation is complete, will explore for new ingredients as well as new ideas.

Thanks

Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

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Attachments: Assessing skin sensitization hazard in mice and men using non-animal tes....pdf; mmc1.xlsx; mmc2.xlsx

Follow Up Flag: Follow up
Flag Status: Flagged

Refer hCLAT data in mmc.xlsx file.

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Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]
Sent: Friday, March 31, 2017 12:33 PM
To: Amar Chittiboyina; 'Ikhlas Khan'; 'CRISTINA AVONTO'; Shabana Khan
Cc: Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Hello Amar,

Thank you for the update on the project status. We have some questions and requests for the further directions of our joint projects:

- 1) Following our last meeting, our understanding was that you have hCLAT and HTS-DCYA results as well as Cys-DPRA and Lys-DPRA results for the 24 fragrance allergens. However, in the "Progress Report_Set1_24 Ingredients.doc" report provided on March 9th, only Cys-DPRA and Lys-DPRA results for the 24 fragrance allergens are provided. We would like to see if you can update the report with hCLAT and HTS-DCYA results and also KeratinoSens results at later time.
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Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Friday, March 03, 2017 3:45 PM
To: Sadrieh, Nakissa; 'Ikhlas Khan'
Cc: 'CRISTINA AVONTO'; Moghaddam, Sarvin; Vukmanovic, Stanislav; Shabana Khan
Subject: RE: Meeting today

Hello Nakissa,

Thank you for the call and it is helpful for us to identify the priorities and goals. As you suggested, we will schedule the teleconference calls for every two months to abreast our research efforts.

In addition to the DPRA report on 24 ingredients, the protocols we are implementing at NCNPR is also attached. Let us know if you need any additional information.

Thanks,
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From: Sadrieh, Nakissa [<mailto:Nakissa.Sadrieh@fda.hhs.gov>]
Sent: Friday, February 17, 2017 10:27 AM
To: 'Ikhlas Khan'
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Moghaddam, Sarvin; Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Do you have a telephone number that we can call? We can try to find a conference room, and then call you. Sarvin and Stan will be joining the discussion. if you want to call us, our conference room telephone number is (b) (6). We will likely use that line. thank you.

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
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Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Friday, February 17, 2017 10:05 AM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: Meeting today

Sure, lets do at 4:00 to be safe.

Which number should we call
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From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, February 17, 2017 at 7:07 AM
To: ikhlas <ikhlan@olemiss.edu>
Subject: Meeting today

Ikhlas,

We have had a meeting scheduled at 2 pm on the other campus, and I won't be back till 3:30. The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

Tsai, Victoria

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Hello Sarvin,

My apologies. Last week, I was tied up with time-sensitive, employee development issues. Certainly can be done. What do mean by % difference between NCNPR and literature results? If you meant number to number for each compound, I don't think it is good idea. Because, the assays we are using are sensitive and the number(s) depended on purity of the compound. And, most of the compounds purity depends on sample age, how they are stored etc.

Thanks,
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I appreciate if you let me know what you think of my email below?

Thank you
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Hi Amar,

We were wondering if you can add extra columns to the existing columns (in the attached file) and include the literature results that you are evaluating your in-house results against and also the % difference between your results and the available literature results.

Thank you,

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Refer hCLAT data in mmc.xlsx file.

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Sent from Amar's iPhone

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P: Positive; N: negative; * The result classification is based on two out of three in-house experiments, however, the

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Tsai, Victoria

From: Amar Chittiboyina <amar@olemiss.edu>
Sent: Friday, May 05, 2017 2:36 PM
To: Moghaddam, Sarvin
Subject: RE: Meeting today
Attachments: Alternative Methods_Binary Classification_Set1.xlsx

Follow Up Flag: Follow up
Flag Status: Flagged

Categories: Red Category

Hello Sarvin,

As you requested, along with data produced at NCNPR with alternative methods (DCYA, DPRA, KeratinoSens and hCLAT), the literature data is also included. The evaluation with KeratinoSens is ongoing and will pass onto you, once it is ready. Let us know if you need any additional information and looking forward for the conference call. Have a good weekend.
Amar

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Anytime tomorrow will work for me.
thanks

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
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Subject: RE: Meeting today

I am already tied up until 2 CST. Suggest me some other time or day. Thanks, Amar

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To: AMAR GOPAL CHITTIBOYINA
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I just noticed you have 2 central time. I might have a meeting at 3 o'clock our time.
So Maybe earlier?

From: AMAR GOPAL CHITTIBOYINA [<mailto:amar@olemiss.edu>]
Sent: Wednesday, May 03, 2017 12:32 PM
To: Moghaddam, Sarvin
Subject: Re: Meeting today

I will call you for a quick chat. So that both of us on same page. May be around 2:00 pm CST.
Amar

Sent from Amar's iPhone

On May 3, 2017, at 9:51 AM, Moghaddam, Sarvin <Sarvin.Moghaddam@fda.hhs.gov> wrote:

Hi Amar,
No worries at all and thanks for getting back to me. We all have our busy days!
I understand your concern about % difference, what do you think about just having the reference
"positive" or "negative" classification. Please let me know what you think or please feel free to call me if
you think it is easier.

Thank you
Sarvin
(240)402-1154

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Attachments: Set2_24 Compounds.xlsx; Common plant allergens_02202017.xlsx; Protocol_DPRA.DOCX; Protocol_hCLAT.DOCX; Protocol_HTS-DCYA.DOCX; Protocol_NMR-DCYA_.docx; Progress Report_Set1_24 Ingredients.docx

Follow Up Flag: Follow up
Flag Status: Flagged

Categories: Red Category

Hello Nakissa,

Thank you for the call and it is helpful for us to identify the priorities and goals. As you suggested, we will schedule the teleconference calls for every two months to abreast our research efforts.

In addition to the DPRA report on 24 ingredients, the protocols we are implementing at NCNPR is also attached. Let us know if you need any additional information.

Thanks,
Amar

From: Sadrieh, Nakissa [mailto:Nakissa.Sadrieh@fda.hhs.gov]
Sent: Friday, February 17, 2017 10:27 AM
To: 'Ikhlas Khan'
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Moghaddam, Sarvin; Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Do you have a telephone number that we can call? We can try to find a conference room, and then call you. Sarvin and Stan will be joining the discussion. if you want to call us, our conference room telephone number is (b) (6) We will likely use that line. thank you.

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Friday, February 17, 2017 10:05 AM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: Meeting today

Sure, lets do at 4:00 to be safe.
Which number should we call
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, February 17, 2017 at 7:07 AM
To: ikhlas <ikhlan@olemiss.edu>
Subject: Meeting today

Ikhlas,

We have had a meeting scheduled at 2 pm on the other campus, and I won't be back till 3:30. The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

Tsai, Victoria

From: Vukmanovic, Stanislav
Sent: Thursday, July 20, 2017 8:52 AM
To: Amar Chittiboyina
Subject: RE: COI

Hi Amar,

Yes, this is fine.

Thanks,

Stan

From: Amar Chittiboyina [mailto:amar@olemiss.edu]
Sent: Wednesday, July 19, 2017 6:15 PM
To: Vukmanovic, Stanislav
Subject: COI

Dear Stan,

As a part of submission, every author needs to disclose the conflict of interest. I went ahead and filled the form on your name. Can you cross-check and send it back to me? Thanks, Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

Tsai, Victoria

From: Ikhlas Khan <ikhan@olemiss.edu>
Sent: Wednesday, August 24, 2016 11:35 PM
To: Sadrieh, Nakissa
Cc: Katz, Linda; Welch, Cara; AMAR GOPAL CHITTIBOYINA; Vukmanovic, Stanislav; Verma, Rajeshwar *
Subject: Re: Cosmetic project

Dear Nakissa

The purpose of this ppt was exactly what you are asking. We collected all the topics from past and what we should do in future. We will prepare all what you asked and we will submit reports as you suggested.

Keratinosens agreement has been approved by the university after a long struggle but cells should be here soon.

We will be in touch soon

ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, August 24, 2016 at 3:42 PM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "Katz, Linda" <Linda.Katz@fda.hhs.gov>, Cara Welch <Cara.Welch@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>, "Verma, Rajeshwar *" <Rajeshwar.Verma@fda.hhs.gov>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Subject: RE: Cosmetic project

Dear Ikhlas,

Thank you for sending me the updated presentation about the ongoing projects in the lab. I met with scientists in our division to discuss your materials, so that we can better focus our joint efforts in the future. The following is a summary of our discussion:

1. We would like to see your deliverable in the form of a report, rather than a Powerpoint presentation. This means that the report would contain the usual contents, such as introduction, materials and methods, results and a discussion and conclusion, highlighting next steps. We are asking for a report because we do not see clear questions and goals for many of the projects in the presentation. We are also missing important methodological details. Therefore, we would like to request a detailed report, written in a word format.
2. Currently our interest is focused on the skin sensitization-related projects. Therefore, please prepare a separate report for the botanicals work that has been completed, and a separate report on the ongoing and planned skin sensitization projects.
3. Please provide more detail about your in silico models and your WoE determinations, since we were not able to fully understand the findings in the Powerpoint slides that you sent us.
4. Please update us on the status of two projects that we discussed during the past year (attached): the Keratinosens and the 26 fragrance testing using ECVAM validated methods. We only saw the results of the h-CLAT data for some of the chemicals in your presentation- have you not done the studies using the DPRA method, which you had previously used in your past projects on botanicals?
5. What is the status on Keratinosens license agreement?

Thank you in advance for your responses.

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Wednesday, August 17, 2016 9:33 AM
To: Sadrieh, Nakissa
Cc: Katz, Linda; Welch, Cara; AMAR GOPAL CHITTIBOYINA
Subject: Cosmetic project

Dear Nakissa

Attached, please find slides which can give an overview of our past activities and outline of future work. We can discuss this by conference call or during your visit that you were planning. We almost have Keratinosense cells, gone through the hurdles and hope we can receive it in couple of weeks. We do appreciate continuous support from OCAC and hope to continue improving collaboration in future.

IK

Tsai, Victoria

From: Ikhlas Khan <ikhan@olemiss.edu>
Sent: Wednesday, April 06, 2016 5:06 PM
To: Vukmanovic, Stanislav; Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle; SHABANA I KHAN
Subject: Re: keratinosens

Dear Nakissa and Stan

After making several rounds of conference call with different departments at University and company in Switzerland, its clear that company won't change their policy and University can't accept their conditions.

Nut after talking to our Lawyer at the university, he suggested some other mechanism which probably will work. It means we can have cell lines in near future.
I will keep you posted.

IK

P.S: Nakissa, I assume you all set for the trip, here is my cell# (b) (6)

From: Ikhlas Khan <ikhan@olemiss.edu>
Date: Wednesday, February 17, 2016 at 1:54 PM
To: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>, shabana <skhan@olemiss.edu>
Subject: Re: keratinosens

Dear Stan

Just wanted to give update about the cell line. As you can imagine, we are still working through the university system. Going back and forth with the agreement. Hope I will have some good news soon
IK

From: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Date: Monday, January 25, 2016 at 12:30 PM
To: Ikhlas Khan <ikhan@olemiss.edu>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>, shabana <skhan@olemiss.edu>
Subject: RE: keratinosens

Great! Thank you so much.

Best,

Stan

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Monday, January 25, 2016 1:26 PM
To: Vukmanovic, Stanislav; Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle; SHABANA I KHAN
Subject: Re: keratinosens

Hi Stan

We had a day off last Friday but it was not too bad. We had back and forth email exchange with the company and they send us transfer agreement to fill. Last week we send them that. We are waiting to hear from them. Once approved, they will send us invoice and we have to deal with our purchasing department.
we will keep you posted
IK

From: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Date: Monday, January 25, 2016 at 12:13 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>
Subject: RE: keratinosens

Hi Ikhlas,

I hope you had nice holidays and not as much snowstorm as we had few days ago.

I just wanted to touch base and see whether by now you may have an idea how easy (or difficult) will it be for you to acquire the license for Keratinocyte™ assay.

Please, let us know any updates you may have.

Best,

Stan

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Friday, December 11, 2015 10:56 AM
To: Sadrieh, Nakissa; Vukmanovic, Stanislav
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle
Subject: Re: keratinosens

Dear Nakissa

We can call you at your office# or you can give a number to call. I assume it's 2:00 CST it's 2 EST your time I will request to delay it to 3:EST or 2:30 EST
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, December 11, 2015 at 9:52 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>
Subject: RE: keratinosens

Ikhlas, are we still talking at 2 pm today? What telephone number would you like us to call, or are you calling us? Thanks.

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Thursday, December 10, 2015 12:00 PM
To: Vukmanovic, Stanislav; Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle
Subject: Re: keratinosens

Thanks, yes it will be helpful to us too
ik

From: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Date: Thursday, December 10, 2015 at 10:45 AM
To: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, Ikhlas Khan <ikhan@olemiss.edu>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>
Subject: RE: keratinosens

Dear Ikhlas and Amar,

In order to have a more productive discussion tomorrow, it may be a good idea to share with you the preliminary plan of the project that we would like to be performed. That way the discussion can be more informative. Your input in design is welcome.

Best,

Stan

From: Sadrieh, Nakissa
Sent: Thursday, December 10, 2015 11:01 AM
To: Ikhlas Khan

Cc: Vukmanovic, Stanislav; AMAR GOPAL CHITTIBOYINA

Subject: Re: keratinosens

Tomorrow is better for me. Maybe we can talk sometime after 2 pm? I will check with my calendar and confirm. Thanks.

From: Ikhlas Khan <ikhlan@olemiss.edu>

Date: December 10, 2015 at 10:31:28 AM EST

To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>

Cc: Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>

Subject: Re: keratinosens

Importance: High

Dear Nakissa

I am back in office and had discussion with Amar and others. Please let me know when you have time for discussion. We are available this afternoon and also tomorrow. If it does not work for you, please suggest for next week

Thanks

IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Wednesday, December 2, 2015 at 8:20 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>

Subject: Re: keratinosens

Thank you Ikhlas. Once I hear from you, we can discuss our proposed study.

From: Ikhlas Khan <ikhlan@olemiss.edu>

Date: December 2, 2015 at 9:18:38 PM EST

To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>

Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>

Subject: Re: keratinosens

Dear Nakissa

We did gather information and tried keratinosens assay but than we were told to focus on H-Clat. We have H-Clat right now that we are using for known compounds but we will be happy to bring Keratinosens assay on board.

I will check the status and let you know.

IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Wednesday, December 2, 2015 at 5:13 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>

Subject: keratinosens

Hi Ikhlas,

I hope that you are well. I am writing to ask you whether U Miss has the Keratinosens assay up and running? This is one of the assays that we have an interest in, and in past discussions, I think that U Miss had mentioned that the assay was available for testing cosmetic ingredients. as you know, Stan Vukmanovic on my staff is working on the immunogenicity of fragrance allergens, and we are hoping to have a battery of tests set up both here and at U Miss, that would allow us to assess the sensitization potential of cosmetic ingredients. I have cc'ed Stan on this email, so that he may be involved in our conversation.

Please let me know if you are in a position to use the Keratinosens assay, because we are interested in a study design that we have developed, to test some fragrance allergens. Thank you.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

Tsai, Victoria

From: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Sent: Wednesday, June 14, 2017 8:38 PM
To: Sadrieh, Nakissa
Cc: Vukmanovic, Stanislav
Subject: Re: Manuscript on 24 fragrance ingredients

Thanks a lot, will wait for your input

Sent from Amar's iPhone

On Jun 14, 2017, at 7:30 PM, Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov> wrote:

Hello Amar,

I am sorry that I let this fall through the cracks. Stan is in vacation now, so I will try to review this and when Stan comes back, he can also review the manuscript. We will send this back by the end of June if that's OK.

From: Amar Chittiboyina <amar@olemiss.edu>
Date: June 14, 2017 at 8:26:17 PM EDT
To: Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>, Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Subject: RE: Manuscript on 24 fragrance ingredients

Hello Nakissa,
Did you get a chance to review the contents on stability and reactivity of 24 ingredients? Thanks, -Amar

Amar Chittiboyina, PhD
Assistant Director, NCNPR
311H TCRC West, School of Pharmacy, University, MS 38655
[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)
pharmacy.olemiss.edu/ncnpr/

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Wednesday, May 31, 2017 4:48 PM
To: Sadrieh, Nakissa (Nakissa.Sadrieh@fda.hhs.gov); Stanislav.Vukmanovic@fda.hhs.gov
Cc: Ikhlas A. Khan (ikh@olemiss.edu); Cristina Avonto (cavonto@olemiss.edu)
Subject: Manuscript on 24 fragrance ingredients

Dear Nakissa and Stan,
Draft on stability and reactivity of 24 fragrance ingredients using in-chemico (DCYA) method is attached. Please review the manuscript and let us know when we can submit it to toxicology journals and your input is very valuable. At this point, we are thinking to send it to Toxicology In vitro. If you see any other journal as a better fit, let us know.
Thanks a lot,

Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

Tsai, Victoria

From: Ikhlas Khan <ikhan@olemiss.edu>
Sent: Friday, February 17, 2017 11:32 AM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Moghaddam, Sarvin; Vukmanovic, Stanislav; Jennifer S. Taylor
Subject: Re: Meeting today

Jennifer will try to get conference call# shortly

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, February 17, 2017 at 10:27 AM
To: ikhlas <ikhan@olemiss.edu>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>, "Moghaddam, Sarvin" <Sarvin.Moghaddam@fda.hhs.gov>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Subject: RE: Meeting today

Do you have a telephone number that we can call? We can try to find a conference room, and then call you. Sarvin and Stan will be joining the discussion. if you want to call us, our conference room telephone number is (b) (6) We will likely use that line. thank you.

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Friday, February 17, 2017 10:05 AM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: Meeting today

Sure, lets do at 4:00 to be safe.
Which number should we call
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, February 17, 2017 at 7:07 AM
To: ikhlas <ikhlan@olemiss.edu>
Subject: Meeting today

Ikhlas,

We have had a meeting scheduled at 2 pm on the other campus, and I won't be back till 3:30. The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

Tsai, Victoria

From: Vukmanovic, Stanislav
Sent: Tuesday, April 11, 2017 2:49 PM
To: Amar Chittiboyina
Subject: RE: Reprint
Attachments: mechasnisms paper online.pdf

Hi Amar,

Of course, I've been meaning to send it to you, but....
Here it is. Warning, it is immunology heavy.

Let me know if you have questions.

Best,

Stan

From: Amar Chittiboyina [mailto:amar@olemiss.edu]
Sent: Tuesday, April 11, 2017 2:20 PM
To: Vukmanovic, Stanislav
Subject: Reprint

Stan,

Can you share the reprint of your recent article on "Skin sensitizers in cosmetics and beyond: potential multiple mechanisms of action and importance of T-cell assays for *in vitro* screening"? Thanks, Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

Tsai, Victoria

From: Amar Chittiboyina <amar@olemiss.edu>
Sent: Friday, October 14, 2016 5:16 PM
To: Sadrieh, Nakissa; 'Ikhlas Khan'
Cc: Katz, Linda; Welch, Cara; Vukmanovic, Stanislav; Verma, Rajeshwar *
Subject: RE: Cosmetic project
Attachments: Development of fluorescence based highthroughput method for potential sk....docx; Development of NMR based inchemico method.docx; Sensitization potential of Tea Tree Oils.docx; Arbutin Stability 090152016.docx; Collaborative research efforts with CFSAN.docx

Hello Nakissa,

Thank you for the conference call. It is very informative for us to understand the needs of your team at OCAC.

1. As per your guidelines, four documents were prepared. These documents are on development of inchemico methods, investigation on tea tree oil and stability of arbutin.
2. A separate document on list of completed projects and on-going projects is attached
3. Regarding skin sensitization of 26 ingredients, we recently purchased ADMET Predictor 8.0 (<http://www.simulations-plus.com/Products.aspx?plD=13>) to assess the sensitization potential of pre-/pro-haptens. We are using this program to understand the role of putative metabolites on reactivity with DCYA or DPRA.
4. Both DPRA and h-CLAT methods were validated in our lab and 24 pure compounds are being evaluated. We will share the results during our visit on 27th of October.
5. After several hurdles (expected and unexpected), we finally received the cell-line for KeratinoSens and we are testing the controls and assay protocols.

Have a good weekend and we will send the requested slides shortly.

Thank you

Amar

From: Sadrieh, Nakissa [mailto:Nakissa.Sadrieh@fda.hhs.gov]
Sent: Wednesday, August 24, 2016 3:42 PM
To: 'Ikhlas Khan'
Cc: Katz, Linda; Welch, Cara; AMAR GOPAL CHITTIBOYINA; Vukmanovic, Stanislav; Verma, Rajeshwar *; Sadrieh, Nakissa
Subject: RE: Cosmetic project

Dear Ikhlas,

Thank you for sending me the updated presentation about the ongoing projects in the lab. I met with scientists in our division to discuss your materials, so that we can better focus our joint efforts in the future. The following is a summary of our discussion:

1. We would like to see your deliverable in the form of a report, rather than a Powerpoint presentation. This means that the report would contain the usual contents, such as introduction, materials and methods, results and a discussion and conclusion, highlighting next steps. We are asking for a report because we do not see clear questions and goals for many of the projects in the presentation. We are also missing important methodological details. Therefore, we would like to request a detailed report, written in a word format.

2. Currently our interest is focused on the skin sensitization-related projects. Therefore, please prepare a separate report for the botanicals work that has been completed, and a separate report on the ongoing and planned skin sensitization projects.
3. Please provide more detail about your in silico models and your WoE determinations, since we were not able to fully understand the findings in the Powerpoint slides that you sent us.
4. Please update us on the status of two projects that we discussed during the past year (attached): the Keratinosens and the 26 fragrance testing using ECVAM validated methods. We only saw the results of the h-CLAT data for some of the chemicals in your presentation- have you not done the studies using the DPRA method, which you had previously used in your past projects on botanicals?
5. What is the status on Keratinosens license agreement?

Thank you in advance for your responses.

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Wednesday, August 17, 2016 9:33 AM
To: Sadrieh, Nakissa
Cc: Katz, Linda; Welch, Cara; AMAR GOPAL CHITTIBOYINA
Subject: Cosmetic project

Dear Nakissa

Attached, please find slides which can give an overview of our past activities and outline of future work. We can discuss this by conference call or during your visit that you were planning. We almost have Keratinosense cells, gone through the hurdles and hope we can receive it in couple of weeks. We do appreciate continuous support from OCAC and hope to continue improving collaboration in future.

IK

Tsai, Victoria

From: Ikhlas Khan <ikhan@olemiss.edu>
Sent: Thursday, February 18, 2016 11:41 AM
To: Vukmanovic, Stanislav; Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle; SHABANA I KHAN
Subject: Re: keratinosens

Dear Stan

Thanks for asking our input. We completely agree with your approach. As we mentioned in last call we are planning to initiate h-Clat and it will be easy to add DPRA since no agreement is required. Hopefully we have all three assay up and running soon and start screening these known 26 compounds first.

Please let us know if you need further information from us in the meantime we will look in to DPRA assay.

Thanks

IK

From: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Date: Wednesday, February 17, 2016 at 1:29 PM
To: Ikhlas Khan <ikhan@olemiss.edu>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>, shabana <skhan@olemiss.edu>
Subject: RE: keratinosens

Thank you so much for your update. I can imagine that there may be a number of hurdles one has to jump over to get this license. Patience is a virtue...

While we are waiting, there is another project that would like to initiate. Part of this project, too, will require Keratinosens license, but the other parts should be doable. In a nutshell, we would like to start generating independent in vitro data on allergens using validated methods (DPRA, Keratinosens, and h-CLAT). We would like to start with the 26 fragrances (hence the attached proposal talks only about these compounds), but the project would eventually expand to other potential allergens. Could you please have a look at the attached proposal and let us know your thoughts.

Thanks again for all your efforts.

Best,

Stan

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Wednesday, February 17, 2016 1:55 PM
To: Vukmanovic, Stanislav; Sadrieh, Nakissa
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From: Ikhlas Khan <ikhlan@olemiss.edu>

Date: December 2, 2015 at 9:18:38 PM EST

To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>

Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>

Subject: Re: keratinosens

Dear Nakissa

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Date: Wednesday, December 2, 2015 at 5:13 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>

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Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

Tsai, Victoria

From: Ikhlas Khan <ikhan@olemiss.edu>
Sent: Thursday, December 10, 2015 12:00 PM
To: Vukmanovic, Stanislav; Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle
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Tel: 240-402-2194

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From: Vukmanovic, Stanislav
Sent: Thursday, February 18, 2016 12:01 PM
To: Ikhlas Khan; Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle; SHABANA I KHAN
Subject: RE: keratinosens

Thank you so much. We are looking forward to see the results.

Best,

Stan

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Thursday, February 18, 2016 11:41 AM
To: Vukmanovic, Stanislav; Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle; SHABANA I KHAN
Subject: Re: keratinosens

Dear Stan

Thanks for asking our input. We completely agree with your approach. As we mentioned in last call we are planning to initiate h-Clat and it will be easy to add DPRA since no agreement is required. Hopefully we have all three assay up and running soon and start screening these known 26 compounds first.

Please let us know if you need further information from us in the meantime we will look in to DPRA assay.

Thanks

IK

From: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Date: Wednesday, February 17, 2016 at 1:29 PM
To: Ikhlas Khan <ikhan@olemiss.edu>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>, shabana <skhan@olemiss.edu>
Subject: RE: keratinosens

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Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>, shabana <skhan@olemiss.edu>
Subject: RE: keratinosens

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From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Monday, January 25, 2016 1:26 PM
To: Vukmanovic, Stanislav; Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle; SHABANA I KHAN
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Attachments: 26f in vitro project v2.docx

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Sent: Thursday, December 10, 2015 12:00 PM
To: Vukmanovic, Stanislav; Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle
Subject: Re: keratinosens

Thanks, yes it will be helpful to us too
ik

From: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Date: Thursday, December 10, 2015 at 10:45 AM
To: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, Ikhlas Khan <ikhan@olemiss.edu>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>
Subject: RE: keratinosens

Dear Ikhlas and Amar,

In order to have a more productive discussion tomorrow, it may be a good idea to share with you the preliminary plan of the project that we would like to be performed. That way the discussion can be more informative. Your input in design is welcome.

Best,

Stan

From: Sadrieh, Nakissa
Sent: Thursday, December 10, 2015 11:01 AM
To: Ikhlas Khan
Cc: Vukmanovic, Stanislav; AMAR GOPAL CHITTIBOYINA
Subject: Re: keratinosens

Tomorrow is better for me. Maybe we can talk sometime after 2 pm? I will check with my calendar and confirm. Thanks.

From: Ikhlas Khan <ikhan@olemiss.edu>
Date: December 10, 2015 at 10:31:28 AM EST
To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Cc: Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: Re: keratinosens
Importance: High

Dear Nakissa

I am back in office and had discussion with Amar and others. Please let me know when you have time for discussion. We are available this afternoon and also tomorrow. If it does not work for you, please suggest for next week

Thanks

IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, December 2, 2015 at 8:20 PM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: Re: keratinosens

Thank you Ikhlas. Once I hear from you, we can discuss our proposed study.

From: Ikhlas Khan <ikhan@olemiss.edu>
Date: December 2, 2015 at 9:18:38 PM EST
To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>

Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>

Subject: Re: keratinosens

Dear Nakissa

We did gather information and tried keratinosens assay but than we were told to focus on H-Clat. We have H-Clat right now that we are using for known compounds but we will be happy to bring Keratinosens assay on board.

I will check the status and let you know.

IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Wednesday, December 2, 2015 at 5:13 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>

Subject: keratinosens

Hi Ikhlas,

I hope that you are well. I am writing to ask you whether U Miss has the Keratinosens assay up and running? This is one of the assays that we have an interest in, and in past discussions, I think that U Miss had mentioned that the assay was available for testing cosmetic ingredients. as you know, Stan Vukmanovic on my staff is working on the immunogenicity of fragrance allergens, and we are hoping to have a battery of tests set up both here and at U Miss, that would allow us to assess the sensitization potential of cosmetic ingredients. I have cc'ed Stan on this email, so that he may be involved in our conversation.

Please let me know if you are in a position to use the Keratinosens assay, because we are interested in a study design that we have developed, to test some fragrance allergens. Thank you.

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

Tsai, Victoria

From: Vukmanovic, Stanislav
Sent: Monday, January 25, 2016 1:14 PM
To: Ikhlas Khan; Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle
Subject: RE: keratinosens

Hi Ikhlas,

I hope you had nice holidays and not as much snowstorm as we had few days ago.

I just wanted to touch base and see whether by now you may have an idea how easy (or difficult) will it be for you to acquire the license for Keratinocyte™ assay.

Please, let us know any updates you may have.

Best,

Stan

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Friday, December 11, 2015 10:56 AM
To: Sadrieh, Nakissa; Vukmanovic, Stanislav
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle
Subject: Re: keratinosens

Dear Nakissa

We can call you at your office# or you can give a number to call. I assume it's 2:00 CST it's 2 EST your time I will request to delay it to 3:EST or 2:30 EST
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, December 11, 2015 at 9:52 AM
To: Ikhlas Khan <ikhan@olemiss.edu>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>
Subject: RE: keratinosens

Ikhlas, are we still talking at 2 pm today? What telephone number would you like us to call, or are you calling us? Thanks.

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

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Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>
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Cc: Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>

Subject: Re: keratinosens

Importance: High

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Cc: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>

Subject: Re: keratinosens

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Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>

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To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>

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Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

Tsai, Victoria

From: Vukmanovic, Stanislav
Sent: Monday, June 26, 2017 8:16 AM
To: Amar Chittiboyina; Sadrieh, Nakissa
Cc: Ikhlas A. Khan; Cristina Avonto
Subject: RE: Manuscript on 24 fragrance ingredients
Attachments: dcya_fragrances draft 05302017.docx

Dear Amar,

Thank you very much for your draft. I found the topic very interesting and enjoyed reading it. I have made some comments and added a paragraph or so to reflect my thoughts on how these results may be interpreted. I hope that you will find the comments helpful and that added text will be acceptable to you.

Also, Nakissa does not feel she has contributed to this manuscript sufficiently to warrant authorship, but is looking forward to future papers on projects we recently discussed.

Thank you,

Stan

From: Amar Chittiboyina [mailto:amar@olemiss.edu]
Sent: Wednesday, May 31, 2017 5:48 PM
To: Sadrieh, Nakissa; Vukmanovic, Stanislav
Cc: Ikhlas A. Khan; Cristina Avonto
Subject: Manuscript on 24 fragrance ingredients

Dear Nakissa and Stan,

Draft on stability and reactivity of 24 fragrance ingredients using in-chemico (DCYA) method is attached. Please review the manuscript and let us know when we can submit it to toxicology journals and your input is very valuable. At this point, we are thinking to send it to Toxicology In vitro. If you see any other journal as a better fit, let us know.

Thanks a lot,

Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

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pharmacy.olemiss.edu/ncnpr/

Tsai, Victoria

From: Vukmanovic, Stanislav
Sent: Monday, June 26, 2017 10:17 AM
To: Amar Chittiboyina
Subject: RE: Manuscript on 24 fragrance ingredients

Hi Amar,

I will certainly ask Nakissa to reconsider, since you put it that way. When you revise, please send it to me again. If everything is OK, only then I will be able to send it for office clearance (only final docs can be cleared, unfortunately).

Looking forward to hear from you,

Stan

From: Amar Chittiboyina [mailto:amar@olemiss.edu]
Sent: Monday, June 26, 2017 9:44 AM
To: Vukmanovic, Stanislav
Cc: Ikhlas A. Khan
Subject: RE: Manuscript on 24 fragrance ingredients

Stan,
Thank for your critique and valuable suggestions, we will address them meticulously. Would you like to re-review after incorporating the changes? Is it cleared from your side? Shall we go ahead and submit to journal?

It's not question of how much is each contributed, rather we want to reflect as a "team" to address the safety concerns of fragrance ingredients and Nakissa is fully involved in our research activities. Can you ask her to re-consider the decision?

Thanks,
Amar

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pharmacy.olemiss.edu/ncnpr/

From: Vukmanovic, Stanislav [mailto:Stanislav.Vukmanovic@fda.hhs.gov]
Sent: Monday, June 26, 2017 7:16 AM
To: Amar Chittiboyina; Sadrieh, Nakissa
Cc: Ikhlas A. Khan; Cristina Avonto
Subject: RE: Manuscript on 24 fragrance ingredients

Dear Amar,

Thank you very much for your draft. I found the topic very interesting and enjoyed reading it. I have made some comments and added a paragraph or so to reflect my thoughts on how these results may be interpreted. I hope that you will find the comments helpful and that added text will be acceptable to you.

Also, Nakissa does not feel she has contributed to this manuscript sufficiently to warrant authorship, but is looking forward to future papers on projects we recently discussed.

Thank you,

Stan

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]

Sent: Wednesday, May 31, 2017 5:48 PM

To: Sadrieh, Nakissa; Vukmanovic, Stanislav

Cc: Ikhlas A. Khan; Cristina Avonto

Subject: Manuscript on 24 fragrance ingredients

Dear Nakissa and Stan,

Draft on stability and reactivity of 24 fragrance ingredients using in-chemico (DCYA) method is attached. Please review the manuscript and let us know when we can submit it to toxicology journals and your input is very valuable. At this point, we are thinking to send it to Toxicology In vitro. If you see any other journal as a better fit, let us know.

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pharmacy.olemiss.edu/ncnpr/

Tsai, Victoria

From: Vukmanovic, Stanislav
Sent: Thursday, June 29, 2017 10:48 AM
To: Amar Chittiboyina
Cc: Sadrieh, Nakissa
Subject: RE: Manuscript on 24 fragrance ingredients

Hi Amar,

Thanks for the fast turnaround, and for taking care of much of my questions. There are still few issues to be resolved, though. I will list them here for clarity, rather than adding comments to the manuscript.

- 1) In regards to citronellol “erratic data”, reading the paper I understood that degradation data was erratic, not the DCYA data. In any case, could you please also send us the file with supplementary data? I would like to see it, and I think it will be required for clearance, as well.
- 2) I don’t understand why anisyl alcohol was declared positive after degradation. Its DCYA activity (in Fig. 5) is overall very similar to cinnamyl alcohol, hexyl cinnamal, amyl cinammyl alcohol and benzyl benzoate, all of which are declared negative. Although I am judging this based on perceived height of bars (and not actual numbers), some bars look very, very similar (e.g. day 92 anisyl alcohol and day 29 benzyl benzoate). This is why my count for positive was 14 and yours was 15. Please review and reconsider.
- 3) Thank you for your explanation of classification of DCYA reactivity (comment CA10). I still think this information needs to find place in the text for the reader, and not be directed only to me (albeit I appreciate it ☺).
- 4) Finally, with regards to Nakissa’s authorship, I spoke to her about it. While we both understand and appreciate your argument about “team efforts”, she feels that different members of the team contribute to different degrees to various portions of the overall project, and that manuscript authorship should reflect contributions to that particular portion. Hence, she feels it would be unethical from her part to accept the authorship on this paper with no contributions to it. As mentioned earlier, she will be looking forward to the authorship on the projects that she has been involved with. Thanks for understanding.

Please, let me know your thoughts on the issues above. These need to be resolved before I send paper for clearance.

Thanks,

Stan

From: Amar Chittiboyina [mailto:amar@olemiss.edu]
Sent: Wednesday, June 28, 2017 5:56 PM
To: Vukmanovic, Stanislav
Subject: RE: Manuscript on 24 fragrance ingredients

Dear Stan,

Revised copy with our responses to your concerns is attached. Once you are satisfied, go ahead and submit for your office clearance. Will be waiting for your e-mail before we submit to journal.

Thanks,

Amar

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To: Amar Chittiboyina

Subject: RE: Manuscript on 24 fragrance ingredients

Hi Amar,

I will certainly ask Nakissa to reconsider, since you put it that way. When you revise, please send it to me again. If everything is OK, only then I will be able to send it for office clearance (only final docs can be cleared, unfortunately).

Looking forward to hear from you,

Stan

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]

Sent: Monday, June 26, 2017 9:44 AM

To: Vukmanovic, Stanislav

Cc: Ikhlas A. Khan

Subject: RE: Manuscript on 24 fragrance ingredients

Stan,

Thank for your critique and valuable suggestions, we will address them meticulously. Would you like to re-review after incorporating the changes? Is it cleared from your side? Shall we go ahead and submit to journal?

It's not question of how much is each contributed, rather we want to reflect as a "team" to address the safety concerns of fragrance ingredients and Nakissa is fully involved in our research activities. Can you ask her to re-consider the decision?

Thanks,
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From: Vukmanovic, Stanislav [<mailto:Stanislav.Vukmanovic@fda.hhs.gov>]

Sent: Monday, June 26, 2017 7:16 AM

To: Amar Chittiboyina; Sadrieh, Nakissa

Cc: Ikhlas A. Khan; Cristina Avonto

Subject: RE: Manuscript on 24 fragrance ingredients

Dear Amar,

Thank you very much for your draft. I found the topic very interesting and enjoyed reading it. I have made some comments and added a paragraph or so to reflect my thoughts on how these results may be interpreted. I hope that you will find the comments helpful and that added text will be acceptable to you.

Also, Nakissa does not feel she has contributed to this manuscript sufficiently to warrant authorship, but is looking forward to future papers on projects we recently discussed.

Thank you,

Stan

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]

Sent: Wednesday, May 31, 2017 5:48 PM

To: Sadrieh, Nakissa; Vukmanovic, Stanislav

Cc: Ikhlas A. Khan; Cristina Avonto

Subject: Manuscript on 24 fragrance ingredients

Dear Nakissa and Stan,

Draft on stability and reactivity of 24 fragrance ingredients using in-chemico (DCYA) method is attached. Please review the manuscript and let us know when we can submit it to toxicology journals and your input is very valuable. At this point, we are thinking to send it to Toxicology In vitro. If you see any other journal as a better fit, let us know.

Thanks a lot,

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pharmacy.olemiss.edu/ncnpr/

Tsai, Victoria

From: Vukmanovic, Stanislav
Sent: Wednesday, July 05, 2017 10:00 AM
To: Amar Chittiboyina
Subject: RE: Manuscript on 24 fragrance ingredients

Thank you, Amar. I have sent the MS for office clearance, and will let you know when the process is completed.

Stan

From: Amar Chittiboyina [mailto:amar@olemiss.edu]
Sent: Friday, June 30, 2017 4:21 PM
To: Vukmanovic, Stanislav
Cc: Sadrieh, Nakissa; Ikhlas A. Khan; Cristina Avonto
Subject: RE: Manuscript on 24 fragrance ingredients

Hi Stan,

See below for our response to your queries (highlighted in red). To make it easy, previous track changes were accepted.

- 1) In regards to citronellol “erratic data”, reading the paper I understood that degradation data was erratic, not the DCYA data. In any case, could you please also send us the file with supplementary data? I would like to see it, and I think it will be required for clearance, as well.

Supplementary data file is attached.

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Due to increase in reactivity with minimal standard deviation, we have included Anisyl alcohol. You are correct, and just to avoid further confusion, we have omitted the Anisyl alcohol from the list and revised manuscript accordingly.

- 3) Thank you for your explanation of classification of DCYA reactivity (comment CA10). I still think this information needs to find place in the text for the reader, and not be directed only to me (albeit I appreciate it ☺).

Agree with you and you raised very good point. Revised the manuscript accordingly.

- 4) Finally, with regards to Nakissa’s authorship, I spoke to her about it. While we both understand and appreciate your argument about “team efforts”, she feels that different members of the team contribute to different degrees to various portions of the overall project, and that manuscript authorship should reflect contributions to that particular portion. Hence, she feels it would be unethical from her part to accept the authorship on this paper with no contributions to it. As mentioned earlier, she will be looking forward to the authorship on the projects that she has been involved with. Thanks for understanding.

We respect her decision and looking forward to work with you closely. Two other manuscripts are in draft stage and we would like to see Nakissa as an author.

Thank you and have a good, long weekend.

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From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
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Sent: Wednesday, May 31, 2017 5:48 PM

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Cc: Ikhlas A. Khan; Cristina Avonto

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Tsai, Victoria

From: Vukmanovic, Stanislav
Sent: Wednesday, July 05, 2017 10:22 AM
To: Amar Chittiboyina
Subject: RE: Manuscript on 24 fragrance ingredients

Hi Amar,

Just got word from Linda about the clearance. She said she will try to get it done by the end of the month. There is an ICCR meeting in Brasil that she is attending next week, so this slows everything else down.

I hope this is OK with you?

Stan

From: Amar Chittiboyina [mailto:amar@olemiss.edu]
Sent: Friday, June 30, 2017 4:21 PM
To: Vukmanovic, Stanislav
Cc: Sadrieh, Nakissa; Ikhlas A. Khan; Cristina Avonto
Subject: RE: Manuscript on 24 fragrance ingredients

Hi Stan,

See below for our response to your queries (highlighted in red). To make it easy, previous track changes were accepted.

- 1) In regards to citronellol “erratic data”, reading the paper I understood that degradation data was erratic, not the DCYA data. In any case, could you please also send us the file with supplementary data? I would like to see it, and I think it will be required for clearance, as well.

Supplementary data file is attached.

- 2) I don’t understand why anisyl alcohol was declared positive after degradation. Its DCYA activity (in Fig. 5) is overall very similar to cinnamyl alcohol, hexyl cinnamal, amyl cinammyl alcohol and benzyl benzoate, all of which are declared negative. Although I am judging this based on perceived height of bars (and not actual numbers), some bars look very, very similar (e.g. day 92 anisyl alcohol and day 29 benzyl benzoate). This is why my count for positive was 14 and yours was 15. Please review and reconsider.

Due to increase in reactivity with minimal standard deviation, we have included Anisyl alcohol. You are correct, and just to avoid further confusion, we have omitted the Anisyl alcohol from the list and revised manuscript accordingly.

- 3) Thank you for your explanation of classification of DCYA reactivity (comment CA10). I still think this information needs to find place in the text for the reader, and not be directed only to me (albeit I appreciate it ☺).

Agree with you and you raised very good point. Revised the manuscript accordingly.

- 4) Finally, with regards to Nakissa’s authorship, I spoke to her about it. While we both understand and appreciate your argument about “team efforts”, she feels that different members of the team contribute to different degrees to various portions of the overall project, and that manuscript authorship should reflect contributions to

that particular portion. Hence, she feels it would be unethical from her part to accept the authorship on this paper with no contributions to it. As mentioned earlier, she will be looking forward to the authorship on the projects that she has been involved with. Thanks for understanding.

We respect her decision and looking forward to work with you closely. Two other manuscripts are in draft stage and we would like to see Nakissa as an author.

Thank you and have a good, long weekend.

Amar

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Wednesday, June 28, 2017 5:56 PM
To: Vukmanovic, Stanislav
Subject: RE: Manuscript on 24 fragrance ingredients

Dear Stan,

Revised copy with our responses to your concerns is attached. Once you are satisfied, go ahead and submit for your office clearance. Will be waiting for your e-mail before we submit to journal.

Thanks,

Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: Vukmanovic, Stanislav [<mailto:Stanislav.Vukmanovic@fda.hhs.gov>]
Sent: Monday, June 26, 2017 9:17 AM
To: Amar Chittiboyina
Subject: RE: Manuscript on 24 fragrance ingredients

Hi Amar,

I will certainly ask Nakissa to reconsider, since you put it that way. When you revise, please send it to me again. If everything is OK, only then I will be able to send it for office clearance (only final docs can be cleared, unfortunately).

Looking forward to hear from you,

Stan

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Monday, June 26, 2017 9:44 AM
To: Vukmanovic, Stanislav
Cc: Ikhlas A. Khan
Subject: RE: Manuscript on 24 fragrance ingredients

Stan,

Thank for your critique and valuable suggestions, we will address them meticulously. Would you like to re-review after incorporating the changes? Is it cleared from your side? Shall we go ahead and submit to journal?

It's not question of how much is each contributed, rather we want to reflect as a "team" to address the safety concerns of fragrance ingredients and Nakissa is fully involved in our research activities. Can you ask her to re-consider the decision?

Thanks,
Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: Vukmanovic, Stanislav [<mailto:Stanislav.Vukmanovic@fda.hhs.gov>]

Sent: Monday, June 26, 2017 7:16 AM

To: Amar Chittiboyina; Sadrieh, Nakissa

Cc: Ikhlas A. Khan; Cristina Avonto

Subject: RE: Manuscript on 24 fragrance ingredients

Dear Amar,

Thank you very much for your draft. I found the topic very interesting and enjoyed reading it. I have made some comments and added a paragraph or so to reflect my thoughts on how these results may be interpreted. I hope that you will find the comments helpful and that added text will be acceptable to you.

Also, Nakissa does not feel she has contributed to this manuscript sufficiently to warrant authorship, but is looking forward to future papers on projects we recently discussed.

Thank you,

Stan

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]

Sent: Wednesday, May 31, 2017 5:48 PM

To: Sadrieh, Nakissa; Vukmanovic, Stanislav

Cc: Ikhlas A. Khan; Cristina Avonto

Subject: Manuscript on 24 fragrance ingredients

Dear Nakissa and Stan,

Draft on stability and reactivity of 24 fragrance ingredients using in-chemico (DCYA) method is attached. Please review the manuscript and let us know when we can submit it to toxicology journals and your input is very valuable. At this point, we are thinking to send it to Toxicology In vitro. If you see any other journal as a better fit, let us know.

Thanks a lot,
Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

Tsai, Victoria

From: Vukmanovic, Stanislav
Sent: Thursday, May 05, 2016 3:17 PM
To: Amar Chittiboyina; Sadrieh, Nakissa
Cc: Ikhlas A. Khan
Subject: RE: Manuscript on Ascaridole, component of tea tree oil
Attachments: CRT_Ascaridole_v3-SV.docx

Hi Amar,

Thank you for the paper, it's good to know what you have been up to. We feel that no one at OCAC has been sufficiently involved in this work to warrant authorship. So, please, go ahead and submit it without us. Thanks for asking, though. I have made some comments in the paper (attached), more of a general science reader type, than a chemistry expert type. I hope they will still be helpful. Good luck with submission!

Best,

Stan

From: Amar Chittiboyina [mailto:amar@olemiss.edu]
Sent: Thursday, May 05, 2016 10:48 AM
To: Sadrieh, Nakissa; Vukmanovic, Stanislav
Cc: Ikhlas A. Khan
Subject: Manuscript on Ascaridole, component of tea tree oil

Dear Nakissa and Stan,

As a part of our ongoing studies on tea tree oil (TTO), we identified possible degradation of ascaridole to reactive species via our *in chemico* method, HTS-DCYA. The attached manuscript is on 'what happens after activation of ascaridole' and implications to TTO. As you know, there are several clinical studies were reported on skin-sensitization of ascaridole and ascaridole containing essential oils and we believe that the identification of such intermediates are very important in assessing the sensitization capacity of TTO.

We are planning to submit it to Chemical Research in Toxicology as a rapid reports. Can you comment on the content and overall findings included in the manuscript? Also, would you suggest us the authors from OCC to include in the manuscript?

Thank you

Amar G. Chittiboyina, PhD
Senior Research Scientist
3040 National Center for Natural Product Research
University, MS 38677
662.915.1572 (off)
662.915.7989 (fax)

Tsai, Victoria

From: Amar Chittiboyina <amar@olemiss.edu>
Sent: Thursday, April 13, 2017 6:08 PM
To: Moghaddam, Sarvin; 'Ikhlas Khan'; 'CRISTINA AVONTO'; 'Shabana Khan'
Cc: Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today
Attachments: hCLAT final results_24compounds_April2017.xlsx; DCYA report of 24 fragrances 04112017.docx

Hello Sarvin,

As per your request, the data on hCLAT and HTS-DCYA is attached. The validation of KeratinoSens is completed in our labs and we are in process of testing these 24 ingredients.

It's the time again for the conference call with your team and suggest us your convenient time.

Thank you and have a good weekend

Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [mailto:Sarvin.Moghaddam@fda.hhs.gov]
Sent: Friday, March 31, 2017 12:33 PM
To: Amar Chittiboyina; 'Ikhlas Khan'; 'CRISTINA AVONTO'; Shabana Khan
Cc: Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Hello Amar,

Thank you for the update on the project status. We have some questions and requests for the further directions of our joint projects:

- 1) Following our last meeting, our understanding was that you have hCLAT and HTS-DCYA results as well as Cys-DPRA and Lys-DPRA results for the 24 fragrance allergens. However, in the "Progress Report_Set1_24 Ingredients.doc" report provided on March 9th, only Cys-DPRA and Lys-DPRA results for the 24 fragrance allergens are provided. We would like to see if you can update the report with hCLAT and HTS-DCYA results and also KeratinoSens results at later time.
- 2) Following some discussion in our group, we would like to expand the project with some new ingredients. Attached, please find the list of ingredients including the initial 24 fragrance allergens for which available results have already been populated.
- 3) Finally, a while ago when we were talking about introducing KeratinoSens assay we discussed a project on delineating the covalent bond-mediated effects from those mediated by reactive oxygen radicals. To refresh our memories, project description is attached. Since KeratinoSens appears to be finally in place, we would like to ask you to make a plan (and send it to us) on carrying this project forward.

Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Friday, March 03, 2017 3:45 PM
To: Sadrieh, Nakissa; 'Ikhlas Khan'
Cc: 'CRISTINA AVONTO'; Moghaddam, Sarvin; Vukmanovic, Stanislav; Shabana Khan
Subject: RE: Meeting today

Hello Nakissa,

Thank you for the call and it is helpful for us to identify the priorities and goals. As you suggested, we will schedule the teleconference calls for every two months to abreast our research efforts.

In addition to the DPRA report on 24 ingredients, the protocols we are implementing at NCNPR is also attached. Let us know if you need any additional information.

Thanks,
Amar

From: Sadrieh, Nakissa [<mailto:Nakissa.Sadrieh@fda.hhs.gov>]
Sent: Friday, February 17, 2017 10:27 AM
To: 'Ikhlas Khan'
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Moghaddam, Sarvin; Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Do you have a telephone number that we can call? We can try to find a conference room, and then call you. Sarvin and Stan will be joining the discussion. if you want to call us, our conference room telephone number is (b) (6) We will likely use that line. thank you.

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Friday, February 17, 2017 10:05 AM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: Meeting today

Sure, lets do at 4:00 to be safe.
Which number should we call

ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Friday, February 17, 2017 at 7:07 AM

To: ikhlas <ikhlan@olemiss.edu>

Subject: Meeting today

Ikhlas,

We have had a meeting scheduled at 2 pm on the other campus, and I won't be back till 3:30. The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

Tsai, Victoria

From: Amar Chittiboyina <amar@olemiss.edu>
Sent: Friday, March 03, 2017 3:45 PM
To: Sadrieh, Nakissa; 'Ikhlas Khan'
Cc: 'CRISTINA AVONTO'; Moghaddam, Sarvin; Vukmanovic, Stanislav; Shabana Khan
Subject: RE: Meeting today
Attachments: Set2_24 Compounds.xlsx; Common plant allergens_02202017.xlsx; Protocol_DPRA.DOCX; Protocol_hCLAT.DOCX; Protocol_HTS-DCYA.DOCX; Protocol_NMR-DCYA_.docx; Progress Report_Set1_24 Ingredients.docx

Hello Nakissa,

Thank you for the call and it is helpful for us to identify the priorities and goals. As you suggested, we will schedule the teleconference calls for every two months to abreast our research efforts.

In addition to the DPRA report on 24 ingredients, the protocols we are implementing at NCNPR is also attached. Let us know if you need any additional information.

Thanks,
Amar

From: Sadrieh, Nakissa [mailto:Nakissa.Sadrieh@fda.hhs.gov]
Sent: Friday, February 17, 2017 10:27 AM
To: 'Ikhlas Khan'
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Moghaddam, Sarvin; Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Do you have a telephone number that we can call? We can try to find a conference room, and then call you. Sarvin and Stan will be joining the discussion. if you want to call us, our conference room telephone number is (b) (6) We will likely use that line. thank you.

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Friday, February 17, 2017 10:05 AM

To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: Meeting today

Sure, lets do at 4:00 to be safe.
Which number should we call
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, February 17, 2017 at 7:07 AM
To: ikhlas <ikhlan@olemiss.edu>
Subject: Meeting today

Ikhlas,

We have had a meeting scheduled at 2 pm on the other campus, and I won't be back till 3:30. The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

Tsai, Victoria

From: Ikhlas Khan <ikhan@olemiss.edu>
Sent: Thursday, March 09, 2017 2:36 PM
To: Sadrieh, Nakissa; AMAR GOPAL CHITTIBOYINA
Cc: Moghaddam, Sarvin; Vukmanovic, Stanislav
Subject: Re: Meeting today

Good. Yes, please let us know when you are ready.
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Thursday, March 9, 2017 at 11:13 AM
To: ikhlas <ikhan@olemiss.edu>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Cc: "Moghaddam, Sarvin" <Sarvin.Moghaddam@fda.hhs.gov>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Subject: FW: Meeting today

Hi Ikhlas,

I just wanted to respond to the email that you just me, and to let you know that we have receive Amar's email, with the attachments. we will get back to you shortly, so that we can get started with the studies.

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Amar Chittiboyina [mailto:amar@olemiss.edu]
Sent: Friday, March 03, 2017 3:45 PM
To: Sadrieh, Nakissa; 'Ikhlas Khan'
Cc: 'CRISTINA AVONTO'; Moghaddam, Sarvin; Vukmanovic, Stanislav; Shabana Khan
Subject: RE: Meeting today

Hello Nakissa,

Thank you for the call and it is helpful for us to identify the priorities and goals. As you suggested, we will schedule the teleconference calls for every two months to abreast our research efforts.

In addition to the DPRA report on 24 ingredients, the protocols we are implementing at NCNPR is also attached. Let us know if you need any additional information.

Thanks,
Amar

From: Sadrieh, Nakissa [<mailto:Nakissa.Sadrieh@fda.hhs.gov>]
Sent: Friday, February 17, 2017 10:27 AM
To: 'Ikhlas Khan'
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Moghaddam, Sarvin; Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Do you have a telephone number that we can call? We can try to find a conference room, and then call you. Sarvin and Stan will be joining the discussion. if you want to call us, our conference room telephone number is (b) (6) We will likely use that line. thank you.

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Friday, February 17, 2017 10:05 AM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: Meeting today

Sure, lets do at 4:00 to be safe.
Which number should we call
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, February 17, 2017 at 7:07 AM
To: ikhlas <ikhan@olemiss.edu>
Subject: Meeting today

Ikhlas,

We have had a meeting scheduled at 2 pm on the other campus, and I won't be back till 3:30. The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

Tsai, Victoria

From: Amar Chittiboyina <amar@olemiss.edu>
Sent: Tuesday, April 11, 2017 2:20 PM
To: Vukmanovic, Stanislav
Subject: Reprint

Stan,

Can you share the reprint of your recent article on “Skin sensitizers in cosmetics and beyond: potential multiple mechanisms of action and importance of T-cell assays for *in vitro* screening”? Thanks, Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: <no subject>
Date: Thursday, April 20, 2017 12:44:38 PM

Hi Nakissa

How are you doing? I will be at CDER giving a talk for OPQ on 25th. Hope we can see each other
ik

From: [Amar Chittiboyina](#)
To: [Sadrieh, Nakissa](#)
Subject: Accepted publication on HTS method for skin sensitizers
Date: Thursday, October 15, 2015 2:26:46 PM
Attachments: [A fluorescence high throughput screening method.pdf](#)

Hi Nakissa,

The work we did with high-throughput method for skin sensitizers has been accepted in Toxicology and Applied Pharmacology.

Thank you

Amar

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#)
Subject: Aloe
Date: Tuesday, November 22, 2016 11:53:29 AM

Sorry here it is

<http://www.msn.com/en-us/money/markets/no-evidence-of-aloe-vera-found-in-the-aloe-vera-at-wal-mart-cvs/ar-AAkBN0x?li=BBnbfcN>

From: Amar Chittiboyina
Date: Tuesday, November 22, 2016 at 10:46 AM
To: ikhlas
Subject: RE: meeting today
[Did you intend to attach article with it? No attachments in the mail.](#)

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Tuesday, November 22, 2016 10:24 AM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA
Subject: Re: meeting today
Hi Nakissa
You were talking about Aloe and here it is
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Thursday, October 27, 2016 at 10:38 AM
To: ikhlas <ikhlan@olemiss.edu>
Subject: meeting today

Ikhlas,

Can I stop by the cafeteria at 12:45? Please start eating, since I need to finish something before I come there. I will eat something in my office before I come there. if I am still hungry, I will get something else to eat to keep you company. thanks.

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Automatic reply: See you soon
Date: Tuesday, August 11, 2015 12:37:41 PM

Dr. Khan is out of office till August 14th, 2015. Please contact Ms. Jennifer jnnfryl@olemiss.edu or by phone 662 915 1090 if you need immediate assistance.

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: call
Date: Friday, February 03, 2017 11:03:03 AM
Importance: High

Hi Nakissa

I tried to call, my office # 662 915 7821

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [Katz, Linda](#); [Welch, Cara](#); [AMAR GOPAL CHITTIBOYINA](#)
Subject: Cosmetic project
Date: Wednesday, August 17, 2016 9:33:47 AM
Attachments: [Update_Cosmetics_08102016.pdf](#)

Dear Nakissa

Attached, please find slides which can give an overview of our past activities and outline of future work. We can discuss this by conference call or during your visit that you were planning. We almost have Keratinosense cells, gone through the hurdles and hope we can receive it in couple of weeks.

We do appreciate continuous support from OCAC and hope to continue improving collaboration in future.

IK

From: [Ikhlas Khan](#)
To: icsb@olemiss.edu
Subject: Draft Agenda
Date: Thursday, February 11, 2016 6:20:23 PM
Attachments: [Agenda 2-11-16_khan\[2\].docx](#)

Please review this draft agenda. Some session title, chairs and additional information needs to be fixed.

Your input will be appreciated

ik

From: [Amar Chittiboyina](#)
To: [Sadrieh, Nakissa](#); [Vukmanovic, Stanislav](#)
Cc: [Ikhlas A. Khan](#)
Subject: FW: Call for Abstracts for the Sixth Annual FDA Foods and Veterinary Medicine Science and Research Conference
Date: Monday, August 15, 2016 5:16:56 PM
Attachments: [6thFVM_SRC_Call4Abstracts.pdf](#)
[OCAC_Potential Abstract_6th FVM_SRC.DOCX](#)

Dear Nakissa,

For the upcoming 6th FVMSRC, attached list of potential abstracts for you to review and consider. Let us know what you would like to submit and we will make the final posters accordingly.

Thanks

Amar

From: Welch, Cara [<mailto:Cara.Welch@fda.hhs.gov>]
Sent: Tuesday, August 09, 2016 12:27 PM
To: ikhan@olemiss.edu; Amar Chittiboyina (amar@olemiss.edu)
Subject: FW: Call for Abstracts for the Sixth Annual FDA Foods and Veterinary Medicine Science and Research Conference

From: CFSAN-ALLHANDS
Sent: Tuesday, August 09, 2016 1:24 PM
To: CFSAN-All Hands
Subject: Call for Abstracts for the Sixth Annual FDA Foods and Veterinary Medicine Science and Research Conference

The FDA Foods and Veterinary Medicine Science and Research Steering Committee is pleased to announce the Call for Abstracts for the Sixth Annual FDA FVM Science and Research Conference "Food Safety, Veterinary Medicine, Nutrition and Cosmetics Research: Meeting the Challenges of a Global Supply Chain." The conference will be held at FDA Headquarters on the White Oak Campus in Silver Spring, Maryland on October 25th and 26th, 2016 and will offer an opportunity to showcase the important research being carried out within the FVM Program and to network with other scientists. We will also have several prominent guest speakers to be announced in the near future. We encourage all staff who conduct FVM-related research to review the Call for Abstracts and consider participating in this event. As always, participation must be approved through your usual supervisory chain.

The deadline for submitting abstracts is August 26, 2016. Please see the attached Call for Abstracts for more details and the link to the electronic submission form, also provided here:

<http://sgiz.mobi/s3/1bc3eacba59f>.

Registration for the conference can be accessed at the following link

<http://sgiz.mobi/s3/c08424562768>.

Periodic updates will be posted on conference intranet site at:

<http://inside.fda.gov:9003/OC/OfficeofFoods/ucm511314.htm>

If you have any questions regarding the Sixth Annual FVM Science and Research Conference, please contact [Dr. Chad Nelson](#) or [Dr. Socrates Trujillo](#).

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: FW: Your Flight Receipt - IKHLAS AHMAD KHAN 11AUG15
Date: Wednesday, August 05, 2015 9:41:43 AM

Dear Nakissa

Here is the itinerary for our visit. We will return at 4 on Thursday. Please let us know when and where we should be on Thursday.

Looking forward to meeting you soon.

My cell# (b) (6)

IK

From: Delta Air Lines <DeltaAirLines@e.delta.com>

Reply-To: Delta Air Lines <support-b99b0asbfpayqkautqx8kqck0h9ws1@e.delta.com>

Date: Thursday, July 23, 2015 at 11:12 AM

To: Ikhlas Khan <ikh@olemiss.edu>

Subject: Your Flight Receipt - IKHLAS AHMAD KHAN 11AUG15

Thanks for choosing Delta. Your Flight is confirmed.



Hello, IKHLAS AHMAD

SkyMiles® #*****089 >

Your Trip Confirmation #: **GL2L45**



Tue, 11AUG	DEPART	ARRIVE
DELTA 2451 MAIN CABIN (V)	MEMPHIS 2:50pm	ATLANTA 5:19pm
DELTA 1738 MAIN CABIN (V)	ATLANTA 6:20pm	WASHINGTON-REAGAN 8:16pm
Thu, 13AUG	DEPART	ARRIVE
DELTA 2401 MAIN CABIN (X)	WASHINGTON-REAGAN 4:00pm	ATLANTA 6:00pm
DELTA 2461 MAIN CABIN (X)	ATLANTA 7:05pm	MEMPHIS 7:33pm

Passenger Info

NAME	FLIGHT	SEAT
IKHLAS AHMAD KHAN	DELTA 2451	25B
SkyMiles #*****089	DELTA 1738	33C
Platinum		

DELTA 240132C

DELTA 246130D

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Flight Receipt

Ticket #: [0062316714956](#)
Place of Issue: Delta.com
Issue Date: 23JUL15
Expiration Date: 23JUL16

METHOD OF PAYMENT

(b) (6)

\$418.70 USD

CHARGES

Air Transportation Charges

Base Fare

\$351.62 USD

Taxes, Fees and Charges

United States - September 11th Security

\$11.20 USD

Fee(Passenger Civil Aviation Security Service Fee) (AY)

United States - Passenger Facility Charge (XF)

\$13.50 USD

United States - Flight Segment Tax (ZP)

\$16.00 USD

United States - Transportation Tax (US)

\$26.38 USD

TICKET AMOUNT

\$418.70 USD


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Fare Details: MEM DL X/ATL DL WAS151.63VA14A0QD DL X/ATL Q27.90 DL MEM172.09XA07A0NA USD351.62END ZP MEMATLDCAATL XF ATL4.5DCA4.5ATL4.5

Checked Bag Allowance

The fees below are based on your original ticket purchase. **If you qualify for free or discounted checked baggage**, this will be taken into account when you check in.

Tue 11 Aug 2015		DELTA: MEM  ATL	
CARRY ON	FIRST	SECOND	
	USD	USD	

FREE

\$25

\$35

Tue 11 Aug 2015

DELTA: ATL → DCA

CARRY ON

FIRST

SECOND

INCLUDED

INCLUDED

INCLUDED

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Thu 13 Aug 2015

DELTA: DCA → ATL

CARRY ON

FIRST

SECOND

FREE

\$25^{USD}\$35^{USD}

Thu 13 Aug 2015

DELTA: ATL → MEM

CARRY ON

FIRST

SECOND

INCLUDED

INCLUDED

INCLUDED

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From: [Ikhlas Khan](#)
To: [Katz, Linda](#)
Cc: [Sadrieh, Nakissa](#); [Milstein, Stanley R](#); [AMAR GOPAL CHITTIBOYINA](#)
Subject: ICSB conference
Date: Monday, September 28, 2015 11:33:42 AM

Dear Linda

Hope everything is fine. Last year we had first time session on cosmetics which Pat organized. We discuss during your visit a possibility to continue this effort. If you all think there is a merit in it please let me know and help us inviting the right people for that particular session on cosmetics.

Conference will be held at Oxford Mississippi, April 11-14, 2016.

www.oxfordicsb.org

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Friday, May 29, 2015 at 2:17 PM
To: Ikhlas Khan <ikhan@olemiss.edu>
Subject: RE: Thanks

Ikhlas,

Thank you as well. The time spent was both informative and useful. I look forward to our future collaboration.

Linda

Linda M. Katz, M.D., M.P.H.

Director, Office of Cosmetics and Colors

Acting Chief Medical Officer Center for Food Safety and Applied Nutrition

Food and Drug Administration

5100 Paint Branch Parkway, HFS-100

College Park, Maryland 20740

240-402-1130 (phone)

301-436-2976 (fax)

linda.katz@fda.hhs.gov

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Friday, May 29, 2015 3:04 PM
To: Katz, Linda
Subject: Thanks

Dear Linda

I would like to thank you for taking time to visit us. I hope you were able to get some information about the program and how we are approaching it. Please feel free to contact us if you need further information. Looking forward to continuing our collaboration.

IK

Ikhlas A. Khan, Ph.D, D. Litt (Hon. Causa)

Asst. Director, NCNPR

Director , FDA Center of Excellence

Director Center for Research in Indian Systems of Medicine (CRISM)

Director of Sino-US TCM Research Center
Research Professor Professor, Dept. of Pharmacognosy
National Center for Natural Products Research School of
Pharmacy University of Mississippi University, MS 38677
USA Tel 662/915/7821 fax 662/915/7989
<http://www.pharmacy.olemiss.edu/ncnpr/index.html>

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: ICSB
Date: Monday, April 04, 2016 11:24:12 AM

Dear Nakissa

I hope you have all travel arrangements done if you need any help please feel free to contact me.

(b) (6)

IK

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: ICSB
Date: Wednesday, February 17, 2016 12:03:31 PM

Hi Nakissa

I believe you are ok with the session. Let me know if you have any suggestion or comment

ik

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: ICSB
Date: Monday, January 25, 2016 5:25:27 PM

Dear Nakissa

Hope you are doing fine and safe in this weather. Please send me the title for your talk. We were able to get David Moyler from Britain as speaker
Here is the list of speakers for your session.

Moderator and Session Chair:

- 1:00-1:30 **Nakissa Sadrich**, Director Cosmetic Division, FDA
1:30-2:00 **Cindy Angerhofer**, Executive Director of Botanical Research, Aveda,
"Ayurvedic Herbs as Sources of Cosmetic Ingredients"
2:00- 2:30 **Amar Chittiboyina**, Senior Research Scientist, University of Mississippi,
"Alternative Testing Methods For Estimating The Skin Sensitization
Potential Of Natural Products"
2:30-3:00 **David Moyler**, Regulatory Consultant, IFRA, IOFI, RIFM and the British
Essential Oils Association, "NCS; Global UN-GHS regulations
implementation", which seems to me to fit the brief"

I have not finalized the time and date yet

Thanks

IK

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [Katz, Linda](#)
Subject: ICSB
Date: Wednesday, November 25, 2015 10:19:05 AM

Dear Nakissa

Linda is working on getting speaker from Europe but I was thinking that you should also give an overview of US regulatory status and efforts being done by OCAC. Please let me know if you are amenable to this idea

IK

Happy Thanks Giving

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: ICSB
Date: Wednesday, October 07, 2015 11:07:58 AM
Importance: High

Dear Nakissa

I was waiting for your response but looks like email never reached you. Do you have sometime this week to discuss about the session.

It should not take long but it will get us started.

Thanks

IK

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: icsb@olemiss.edu
Subject: ICSB
Date: Monday, March 06, 2017 11:15:27 AM

Hi Nakissa

I know you are busy but we need your abstract and title to complete the booklet. I will appreciate if you can forward it to us.

Appreciate it

ik

From: [Amar Chittiboyina](#)
To: [Sadrieh, Nakissa](#); [Vukmanovic, Stanislav](#)
Cc: [Ikhlas A. Khan](#); [Cristina Avonto](#)
Subject: Manuscript on 24 fragrance ingredients
Date: Wednesday, May 31, 2017 5:48:14 PM
Attachments: [dcya_fragrances draft 05302017.docx](#)

Dear Nakissa and Stan,

Draft on stability and reactivity of 24 fragrance ingredients using in-chemico (DCYA) method is attached. Please review the manuscript and let us know when we can submit it to toxicology journals and your input is very valuable. At this point, we are thinking to send it to Toxicology In vitro. If you see any other journal as a better fit, let us know.

Thanks a lot,

Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: [Amar Chittiboyina](#)
To: [Sadrieh, Nakissa](#); [Vukmanovic, Stanislav](#)
Cc: [Ikhlas A. Khan](#)
Subject: Manuscript on Ascaridole, component of tea tree oil
Date: Thursday, May 05, 2016 10:48:32 AM
Attachments: [CRT_Ascaridole_v3.docx](#)
[Supp_Info_v3.pdf](#)

Dear Nakissa and Stan,

As a part of our ongoing studies on tea tree oil (TTO), we identified possible degradation of ascaridole to reactive species via our *in chemico* method, HTS-DCYA. The attached manuscript is on 'what happens after activation of ascaridole' and implications to TTO. As you know, there are several clinical studies were reported on skin-sensitization of ascaridole and ascaridole containing essential oils and we believe that the identification of such intermediates are very important in assessing the sensitization capacity of TTO.

We are planning to submit it to Chemical Research in Toxicology as a rapid reports. Can you comment on the content and overall findings included in the manuscript? Also, would you suggest us the authors from OCC to include in the manuscript?

Thank you

Amar G. Chittiboyina, PhD
Senior Research Scientist
3040 National Center for Natural Product Research
University, MS 38677
662.915.1572 (off)
662.915.7989 (fax)

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#)
Subject: protocols
Date: Thursday, March 09, 2017 12:10:49 PM

Dear Nakissa

Just want to confirm that you received email from Amar about protocols etc. He is having issues, somehow his email is not reaching CFSAN.

Thanks

ik

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: <no subject>
Date: Thursday, April 20, 2017 5:33:14 PM

Hi NAKissa

I am giving a lecture in Botanical Drug Seminar series. I do not have agenda from them yet but if there is a time I will try to meet you. I think Amar is planning to have conference call soon to discuss future work.

IK

From: "Sadrieh, Nakissa"

Date: Thursday, April 20, 2017 at 4:16 PM

To: ikhlas

Subject: RE:

Hi Ikhlas. I used to work in OPS in CDER, which is the previous name for OPQ. Who are you meeting? It would be great if you can find time to meet with us here during your visit.

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]

Sent: Thursday, April 20, 2017 12:44 PM

To: Sadrieh, Nakissa

Subject:

Hi Nakissa

How are you doing? I will be at CDER giving a talk for OPQ on 25th. Hope we can see each other
ik

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [Jennifer S. Taylor](#)
Subject: Re: agenda for the meeting
Date: Wednesday, March 16, 2016 9:04:49 PM

Hi Nakissa

I will send the agenda first thing in the morning. Jennifer can tell you more about hotel and pick up.

Here is the website www.oxfordicsb.org

Ik

Sent from my iPhone

On Mar 16, 2016, at 4:39 PM, Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov> wrote:

Ikhlas,

Hello. Can I please ask you to send me the agenda for the upcoming meeting, as well as the information for the travel, such as which airport I need to fly into, and whether in need to rent a car or will there be a taxi to bring me from the airport to the hotel, and where I should stay (which hotel)? I need to do my travel arrangements, and decide on travel dates and hotels and other things. I also need to send an abstract. Thanks.

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: agenda for the meeting
Date: Thursday, March 17, 2016 10:14:06 PM

Please let me know if you need further information
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Thursday, March 17, 2016 at 6:13 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: ac <jnnfrtyl@olemiss.edu>
Subject: Re: agenda for the meeting

Thank you. I will send the abstract today hopefully. I will try to send my slides by the end of March.

From: Ikhlas Khan <ikhlan@olemiss.edu>
Date: March 16, 2016 at 9:04:48 PM EDT
To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Cc: Jennifer S. Taylor <jnnfrtyl@olemiss.edu>
Subject: Re: agenda for the meeting

Hi Nakissa
I will send the agenda first thing in the morning. Jennifer can tell you more about hotel and pick up.
Here is the website www.oxfordicsb.org
Ik

Sent from my iPhone

On Mar 16, 2016, at 4:39 PM, Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov> wrote:

Ikhlas,
Hello. Can I please ask you to send me the agenda for the upcoming meeting, as well as the information for the travel, such as which airport I need to fly into, and whether in need to rent a car or will there be a taxi to bring me from the airport to the hotel, and where I should stay (which hotel)? I need to do my travel arrangements, and decide on travel dates and hotels and other things. I also need to send an abstract. Thanks.
Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Katz, Linda](#)
Cc: [Sadrieh, Nakissa](#)
Subject: Re: Botanicals workshop at U Miss-Candidates for presentations
Date: Friday, November 20, 2015 11:51:28 AM

Dear Linda

I contacted Laurent a week ago but no response. Do you recommend anyone on regulations from Europe or we should get someone from Associations here in US to speak at ICSB.

Thanks

Ik

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Tuesday, November 10, 2015 at 9:22 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Subject: RE: Botanicals workshop at U Miss-Candidates for presentations

Feel free to contact Laurent Selles – contact information is attached below. Laurent's portfolio includes cosmetics. I am not sure if he deals with botanical issues but I am sure that he can direct you to the correct person if he is not. Feel free to use my name.

Laurent SELLES

Senior Coordinator for International Relations



European Commission

DG for Internal Market, Industry, Entrepreneurship and SMEs
Unit D4 – Health Technology & Cosmetics
BREY 12/28 B-1049 Brussels/Belgium
+32 2 296 34 20 laurent.selles@ec.europa.eu

Linda

From: Sadrieh, Nakissa
Sent: Tuesday, November 10, 2015 10:18 AM
To: 'Ikhlas Khan'
Cc: Katz, Linda; Sadrieh, Nakissa
Subject: RE: Botanicals workshop at U Miss-Candidates for presentations

Hi Ikhlas. I am cc'ing Linda on this email, since I have no interactions with international counterparts. Maybe Linda can provide input regarding your question, since she represents cosmetics on all international activities. Thanks.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Tuesday, November 10, 2015 10:07 AM
To: Sadrieh, Nakissa
Subject: Re: Botanicals workshop at U Miss-Candidates for presentations

Dear Nakissa

Hope you are doing fine. I am back from trips and started working on ICSB agenda. Do you have your counter part in Europe or anyone you know them.
IK

From: Ikhlas Khan <ikhlan@olemiss.edu>
Date: Thursday, October 29, 2015 at 2:17 PM
To: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Subject: Re: Botanicals workshop at U Miss-Candidates for presentations

Dear Nakissa

Thanks for the list we will see who is available and who fits the best according to your suggestions.
Do you know or prefer any European regulator that you would like to invite.
IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, October 28, 2015 at 12:55 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Subject: Botanicals workshop at U Miss-Candidates for presentations

Ikhlas,

I have provided some possible candidates for the botanicals workshop. I am hoping that you or your staff may be able to follow up with some of these candidates, to identify those that can participate and to discuss travel arrangements with the speakers. I am also providing to you the plan for 3 general topics, for the session, as I imagine it:

<!--[if !supportLists]-->1. <!--[endif]-->Isolation, characterization, formulation and manufacturing considerations for botanical used in cosmetic products.

- <!--[if !supportLists]-->2. <!--[endif]-->Safety considerations (clinical and preclinical) for botanicals used in cosmetics (testing as well as adverse events analysis)
- <!--[if !supportLists]-->3. <!--[endif]-->European/international regulatory position on the use of botanicals in cosmetics

The possible candidates are listed below:

ACADEMIC/Safety assessment

<!--[if !supportLists]-->1. <!--[endif]-->**Thomas Re** (L'Oreal USA, Research and Development, Clark, NJ 07066, USA)
Senior Principle Scientist at L'Oreal S.A.

"Review Safety assessment of personal care products/cosmetics and their ingredients" Gerhard J. Nohynek, , Eric Antignac, Thomas Reb, Herve Toutain
Toxicology and Applied Pharmacology 243 (2010) 239–259

(1998 joined Cosmair, Inc which became L'Oreal USA as a Director of the Clinical Safety Group responsible for human safety studies on all L'Oreal products developed in the United States including Maybelline and RedKen brands. Was responsible for Raw Material Toxicology for various product lines and international safety attestations. Serve as an expert witness for legal team as needed. Now Senior Principle Scientist.)

<!--[if !supportLists]-->2. <!--[endif]-->**Judy L Bolton**
Professor, Medicinal Chemistry and Pharmacognosy
833 South Wood Street
Chicago, IL 60612
Email: judyb@uic.edu
Phone: (312) 996-5280
Fax: (312) 996-7107

Pharmacokinetics of prenylated hop phenols in women following oral administration of a standardized extract of hops. 58. Molecular nutrition & food research:2014:10, 1962-9

Biological and chemical standardization of a hop (Humulus lupulus) botanical dietary supplement. 28. Biomedical chromatography : BMC:2014:6, 729-34

SERMs attenuate estrogen-induced malignant transformation of human mammary epithelial cells by upregulating detoxification of oxidative metabolites. 7. Cancer prevention research (Philadelphia, Pa.):2014:5, 505-15

Quinone Methide Bioactivation Pathway: Contribution to Toxicity and/or Cytoprotection?. 18.
Current organic chemistry:2014:1, 61-69

Evaluation of estrogenic activity of licorice species in comparison with hops used in botanicals for menopausal symptoms. 8. PloS one:2013:7, e67947

<!--[if !supportLists]-->3. <!--[endif]-->**Chun-Tao Che, Ph.D.**

Norman R. Farnsworth Professor of Pharmacognosy
Department of Medicinal Chemistry and Pharmacognosy

University of Illinois at Chicago
College of Pharmacy
833 South Wood Street (M/C 781)
Chicago IL 60612-7231

Q.-F. Hu, B. Zhou, J.-M. Huang, Z.-Y. Jiang, X.-Z. Huang, L.-Y. Yang, X.-M. Gao, G.-Y. Yang, and C.T. Che, Cytotoxic Oxepinochromenone and Flavonoids from the Flower Buds of *Rosa rugosa*. J. Nat. Prod. 76: 1866-1871, 2013.

M. Zhao, X. Zhang, Y. Wang, M. Huang, J.-A. Duan, T. Gödecke, K.M. Szymulanska-Ramamurthy, Z. Yin and C.T. Che, Germacranes and m-Menthane from *Illicium lanceolatum*. Molecules 19: 4326-4337, 2014.

C. M. Witt, M. Aickin, D. Cherkin, C.T. Che, C. Elder, A. Flower, R. Hammerschlag, J.-P. Liu, L. Lao, S. Phurrough, C. Ritenbaugh, L.H. Rubin, R. Schnyer, P.M. Wayne, S.R. Withers, Z.X. Bian, J. Young and B.M. Berman, Effectiveness guidance document (EGD) for Chinese medicine trials: A consensus document. Trials 15: 169, 2014. [DOI:10.1186/1745-6215-15-169]

D.-T. Xie, Y.-Q. Wang, Y. Kang, Q.-F. Hu, N.-Y. Su, J.-M. Huang, C.T. Che and J.-X. Guo, Microwave-assisted extraction of bioactive alkaloids from *Stephania sinica*. Sep. Purif. Techol., 130: 173-181, 2014.

F.W.K. Cheung, A.W.N. Leung, W.K. Liu and C.T. Che, Tyrosinase inhibitory activity of a glucosylated hydroxystilbene in mouse Melan-a melanocytes. J. Nat. Prod. 77: 1270-1274, 2014.

<!--[if !supportLists]-->4. <!--[endif]-->**Bela Peethambaran PhD**

Assistant Professor of Biology

Pasted from <<http://www.gradschool.usciences.edu/faculty/peethambaran-bela>>

Dhillon, J., Miller, V., Badiab A., Tang CN., Huynh A., Peethambaran, B. 2014. Apoptosis-inducing potential of *Myrothamnus flabellifolius*, an edible medicinal plant, on human myeloid leukemia HL-60 cells. International Journal of Applied Research in Natural Products, 7:28-32

Tran, L., Naik, A., Koronkiewicz, B., Peethambaran, B. 2014. Epigallocatechin gallate Inhibits Biofilm Production and Attenuates Virulent Factors of Pseudomonas aeruginosa and Psuedomonas fluorescence. Journal of Natural Remedies, 14:106-111

Lindberg, J., Nabbie, F., Milliot, J., Smith, R., M Crisitina Tettamanzi., Peethambaran, B. 2014. 14-3-3λ Affects Production of a Sinapoyl Derivative in Lignin Biosynthesis during Drought Stress in Arabidopsis thaliana. Universal Journal of Plant Sciences, Vol 2, no. 4: 77-85

Smith, R., Peethambaran B., Pontiggio, L., Blumberg, P. 2013. Does a repeated guided-instruction approach with multiple assessments increase student learning of science? Journal of Biological Education , 47: 11-116

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Tel: 240-402-2194

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To: [Katz, Linda](#)
Cc: [Sadrieh, Nakissa](#)
Subject: Re: Botanicals workshop at U Miss-Candidates for presentations
Date: Tuesday, November 10, 2015 10:32:39 AM

Appreciate quick response. I will get in touch and let you know

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Date: Tuesday, November 10, 2015 at 9:22 AM
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Feel free to contact Laurent Selles – contact information is attached below. Laurent's portfolio includes cosmetics. I am not sure if he deals with botanical issues but I am sure that he can direct you to the correct person if he is not. Feel free to use my name.

Laurent SELLES

Senior Coordinator for International Relations



European Commission

DG for Internal Market, Industry, Entrepreneurship and SMEs
Unit D4 – Health Technology & Cosmetics
BREY 12/28 B-1049 Brussels/Belgium
+32 2 296 34 20 laurent.selles@ec.europa.eu

Linda

From: Sadrieh, Nakissa
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Subject: Re: Botanicals workshop at U Miss-Candidates for presentations
Date: Tuesday, November 10, 2015 10:22:53 AM

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Department of Medicinal Chemistry and Pharmacognosy

University of Illinois at Chicago

College of Pharmacy

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INDUSTRY/Manufacturing

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Please let me know what you think. thanks.

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Director, Cosmetics Division

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Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: Botanicals workshop at U Miss-Candidates for presentations
Date: Thursday, October 29, 2015 4:17:14 PM

Dear Nakissa

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Do you know or prefer any European regulator that you would like to invite.

IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, October 28, 2015 at 12:55 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Katz, Linda" <Linda.Katz@fda.hhs.gov>
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Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Katz, Linda](#)
Cc: [Sadrieh, Nakissa](#)
Subject: Re: Botanicals workshop at U Miss-Candidates for presentations
Date: Friday, November 20, 2015 12:10:44 PM

Please. That will be big help
ik

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Friday, November 20, 2015 at 11:08 AM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Subject: RE: Botanicals workshop at U Miss-Candidates for presentations

I will forward the request if you'd like.

[Linda](#)

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Friday, November 20, 2015 11:51 AM
To: Katz, Linda
Cc: Sadrieh, Nakissa
Subject: Re: Botanicals workshop at U Miss-Candidates for presentations

Dear Linda

I contacted Laurent a week ago but no response. Do you recommend anyone on regulations from Europe or we should get someone from Associations here in US to speak at ICSB.

Thanks

Ik

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Tuesday, November 10, 2015 at 9:22 AM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Subject: RE: Botanicals workshop at U Miss-Candidates for presentations

Feel free to contact Laurent Selles – contact information is attached below. Laurent's portfolio includes cosmetics. I am not sure if he deals with botanical issues but I am sure that he can direct you to the correct person if he is not. Feel free to use my name.

Laurent SELLES

Senior Coordinator for International Relations



European Commission

DG for Internal Market, Industry, Entrepreneurship and SMEs
Unit D4 – Health Technology & Cosmetics
BREY 12/28 B-1049 Brussels/Belgium
+32 2 296 34 20 laurent.selles@ec.europa.eu

Linda

From: Sadrieh, Nakissa
Sent: Tuesday, November 10, 2015 10:18 AM
To: 'Ikhlas Khan'
Cc: Katz, Linda; Sadrieh, Nakissa
Subject: RE: Botanicals workshop at U Miss-Candidates for presentations

Hi Ikhlas. I am cc'ing Linda on this email, since I have no interactions with international counterparts. Maybe Linda can provide input regarding your question, since she represents cosmetics on all international activities. Thanks.

Regards,
Nakissa Sadrieh, Ph.D.
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Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Tuesday, November 10, 2015 10:07 AM
To: Sadrieh, Nakissa
Subject: Re: Botanicals workshop at U Miss-Candidates for presentations

Dear Nakissa
Hope you are doing fine. I am back from trips and started working on ICSB agenda.
Do you have your counter part in Europe or anyone you know them.
IK

From: Ikhlas Khan <ikhlan@olemiss.edu>
Date: Thursday, October 29, 2015 at 2:17 PM
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Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: call
Date: Monday, February 13, 2017 5:52:28 PM

Hi Nakissa

Does coming Friday morning 10:15 EST works for you for a conference call.

IK

From: "Sadrieh, Nakissa"

Date: Friday, February 3, 2017 at 11:15 AM

To: ikhlas

Cc: AMAR GOPAL CHITTIBOYINA

Subject: RE: call

My FDA cell phone is 240-994-2874. Thanks for giving me your number too.

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Friday, February 03, 2017 12:12 PM

To: Sadrieh, Nakissa

Cc: AMAR GOPAL CHITTIBOYINA

Subject: Re: call

Yes, that email confused me too. No problem, we can talk next week. It will good to have your cell#

in case. Here is mine (b) (6)

ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Friday, February 3, 2017 at 11:02 AM

To: ikhlas <ikhlan@olemiss.edu>

Subject: RE: call

I am sorry about this. I saw a cancellation sent by Cara yesterday, and I thought that it impacted our meeting. I must have misunderstood. Let's talk next week. I have a new chemist assigned to the work with your lab, so she is catching up with the work already done, and by next week, we can have a more fruitful discussion, about next steps.

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Friday, February 03, 2017 11:26 AM
To: Sadrieh, Nakissa
Subject: Re: call
Importance: High
Lets plan another time. Give me time and date
ik

From: ikhlas <ikhlan@olemiss.edu>
Date: Friday, February 3, 2017 at 10:01 AM
To: "Nakissa.Sadrieh@fda.hhs.gov" <Nakissa.Sadrieh@fda.hhs.gov>
Subject: call
Hi Nakissa
I tried to call, my office # 662 915 7821

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: call
Date: Friday, February 03, 2017 12:12:56 PM

Yes, that email confused me too. No problem, we can talk next week. It will good to have your cell# in case. Here is mine (b) (6)
ik

From: "Sadrieh, Nakissa"
Date: Friday, February 3, 2017 at 11:02 AM
To: ikhlas
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From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: call
Date: Friday, February 03, 2017 11:26:03 AM
Importance: High

Lets plan another time. Give me time and date
ik

From: ikhlas
Date: Friday, February 3, 2017 at 10:01 AM
To: "Nakissa.Sadrieh@fda.hhs.gov"
Subject: call

Hi Nakissa

I tried to call, my office # 662 915 7821

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: call
Date: Tuesday, February 14, 2017 12:34:55 PM

We can do 3 EST on Friday. Let me know if it works for you.
IK

From: "Sadrieh, Nakissa"
Date: Tuesday, February 14, 2017 at 11:31 AM
To: ikhlas
Cc: AMAR GOPAL CHITTIBOYINA
Subject: RE: call

Hi Ikhlas,

10:15 on Friday won't work for me, since I have another meeting scheduled. Would sometime in the afternoon on Friday work for you?

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Monday, February 13, 2017 5:52 PM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA
Subject: Re: call

Hi NAKissa

Does coming Friday morning 10:15 EST works for you for a conference call.

IK

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Date: Friday, February 3, 2017 at 11:15 AM
To: ikhlas <ikhan@olemiss.edu>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: RE: call

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Nakissa Sadrieh, Ph.D.
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Office of Cosmetics and Colors (OCAC)

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Tel: 240-402-2194

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Sent: Friday, February 03, 2017 12:12 PM

To: Sadrieh, Nakissa

Cc: AMAR GOPAL CHITTIBOYINA

Subject: Re: call

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From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Friday, February 03, 2017 11:26 AM

To: Sadrieh, Nakissa

Subject: Re: call

Importance: High

Lets plan another time. Give me time and date
ik

From: ikhlas <ikhlan@olemiss.edu>

Date: Friday, February 3, 2017 at 10:01 AM

To: "Nakissa.Sadrieh@fda.hhs.gov" <Nakissa.Sadrieh@fda.hhs.gov>

Subject: call

Hi Nakissa

I tried to call, my office # 662 915 7821

From: [Ikhlas Khan](#)
To: [Milstein, Stanley R](#)
Cc: [Sadrieh, Nakissa](#)
Subject: Re: CFSAN-OCAC and University of Mississippi Meeting (August 13, 2015)
Date: Sunday, August 16, 2015 5:29:11 PM

Dear Stan

It was great pleasure to meet the group and had a fruitful discussion. Please contact us anytime if you have a question or comment. Looking forward to having a fruitful collaboration

IK

From: "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>
Date: Thursday, August 13, 2015 at 1:58 PM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Subject: CFSAN-OCAC and University of Mississippi Meeting (August 13, 2015)

Dear Dr. Khan (Ikhlas) ---

Good afternoon. Thanks very much (both to yourself as well as to your colleagues from University of Mississippi) for taking the time to visit with us this morning at CFSAN-OCAC and to present to us retrospective results of your investigations, to date, as well as prospective visions of collaborative work projects yet to come. It was enjoyable meeting you and your colleagues, and Dr. Nakissa Sadrieh and I will summarize and report back to Dr. Linda Katz our thoughts about this very productive set of discussions, upon her return to OCAC next week.

Attached, you will find the promised information about the new AOAC International "Cosmetics and Color Additives Community" (which actually includes cosmetics, color additives, and microbiology).

The members of the Community Steering Committee here at CFSAN include:

Alexander Krynitsky, Ph.D. (ORS – Bioanalytical Methods)

Thomas Hammack, Ph.D. (ORS – Microbiological Methods)

Patricia Hansen, Ph.D. (OCAC, ONLDS)

Bhakti Petigara, Ph.D. (OCAC – Color Additives)

Stanley R. Milstein, Ph.D. (OCAC)

At the present time, the "points of contact (POC)" for the Community are: **Drs. Krynitsky, Hammack, and Petigara**, and you can contact them for further information about the upcoming AOAC International Meeting in September, 2015 (Los Angeles, CA); their respective e-mail addresses here at CFSAN are given on the last slide of the .pptx.

I hope that you and/or one of your colleagues from University of Mississippi may have already scheduled attendance at the AOAC International Meeting in Los Angeles and will take the opportunity to engage our new Cosmetics and Color Additives Community while there, with a view towards exploring with members of the Community where additional opportunities for collaboration may lie.

I look forward to continuing the dialogues begun (or continued !) this morning at CFSAN-OCAC.

Best Regards

Stan Milstein

Stanley R. Milstein, Ph.D. (HFS-100)

Acting Deputy Director
Office of Cosmetics and Colors
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
College Park, MD 20740
Stanley.milstein@fda.hhs.gov

From: [Amar Chittiboyina](#)
To: [Sadrieh, Nakissa](#)
Cc: [Ikhlas A. Khan](#); [Dr. Cristina Avonto](#)
Subject: RE: Cosmetic project
Date: Monday, October 17, 2016 3:35:59 PM
Attachments: [Slides cosmetic work NS v3.pptx](#)
[Narrative NS 10172016.docx](#)

Hello Nakissa,

As we assured, both slides and corresponding notes are attached. If you need any additional information or need more slides, do not hesitate to contact us.

Thank you,

Amar

From: Sadrieh, Nakissa [mailto:Nakissa.Sadrieh@fda.hhs.gov]
Sent: Monday, October 17, 2016 1:39 PM
To: 'Amar Chittiboyina'; 'Ikhlas Khan'
Cc: Katz, Linda; Welch, Cara; Vukmanovic, Stanislav; Verma, Rajeshwar *
Subject: RE: Cosmetic project

Thank you Amar for this information. I will go through these, and I look forward to getting the slides as well. I will certainly be in touch with you.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: [Amar Chittiboyina](#)
To: [Sadrieh, Nakissa](#); "[Ikhlas Khan](#)"
Cc: [Katz, Linda](#); [Welch, Cara](#); [Vukmanovic, Stanislav](#); [Verma, Rajeshwar *](#)
Subject: RE: Cosmetic project
Date: Friday, October 14, 2016 5:15:49 PM
Attachments: [Development of fluorescence based highthroughput method for potential sk....docx](#)
[Development of NMR based inchemico method.docx](#)
[Sensitization potential of Tea Tree Oils.docx](#)
[Arbutin Stability 090152016.docx](#)
[Collaborative research efforts with CFSAN.docx](#)

Hello Nakissa,

Thank you for the conference call. It is very informative for us to understand the needs of your team at OCAC.

1. As per your guidelines, four documents were prepared. These documents are on development of inchemico methods, investigation on tea tree oil and stability of arbutin.
2. A separate document on list of completed projects and on-going projects is attached
3. Regarding skin sensitization of 26 ingredients, we recently purchased ADMET Predictor 8.0 (<http://www.simulations-plus.com/Products.aspx?PID=13>) to assess the sensitization potential of pre-/pro-haptens. We are using this program to understand the role of putative metabolites on reactivity with DCYA or DPRA.
4. Both DPRA and h-CLAT methods were validated in our lab and 24 pure compounds are being evaluated. We will share the results during our visit on 27th of October.
5. After several hurdles (expected and unexpected), we finally received the cell-line for KeratinoSens and we are testing the controls and assay protocols.

Have a good weekend and we will send the requested slides shortly.

Thank you

Amar

From: Sadrieh, Nakissa [mailto:Nakissa.Sadrieh@fda.hhs.gov]
Sent: Wednesday, August 24, 2016 3:42 PM
To: 'Ikhlas Khan'
Cc: Katz, Linda; Welch, Cara; AMAR GOPAL CHITTIBOYINA; Vukmanovic, Stanislav; Verma, Rajeshwar *; Sadrieh, Nakissa
Subject: RE: Cosmetic project

Dear Ikhlas,

Thank you for sending me the updated presentation about the ongoing projects in the lab. I met with scientists in our division to discuss your materials, so that we can better focus our joint efforts in the future. The following is a summary of our discussion:

1. We would like to see your deliverable in the form of a report, rather than a Powerpoint presentation. This means that the report would contain the usual contents, such as introduction, materials and methods, results and a discussion and conclusion, highlighting next steps. We are asking for a report because we do not see clear questions and goals for many of the projects in the presentation. We are also missing

important methodological details. Therefore, we would like to request a detailed report, written in a word format.

2. Currently our interest is focused on the skin sensitization-related projects. Therefore, please prepare a separate report for the botanicals work that has been completed, and a separate report on the ongoing and planned skin sensitization projects.
3. Please provide more detail about your in silico models and your WoE determinations, since we were not able to fully understand the findings in the Powerpoint slides that you sent us.
4. Please update us on the status of two projects that we discussed during the past year (attached): the Keratinosens and the 26 fragrance testing using ECVAM validated methods. We only saw the results of the h-CLAT data for some of the chemicals in your presentation- have you not done the studies using the DPRA method, which you had previously used in your past projects on botanicals?
5. What is the status on Keratinosens license agreement?

Thank you in advance for your responses.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Wednesday, August 17, 2016 9:33 AM
To: Sadrieh, Nakissa
Cc: Katz, Linda; Welch, Cara; AMAR GOPAL CHITTIBOYINA
Subject: Cosmetic project

Dear Nakissa

Attached, please find slides which can give an overview of our past activities and outline of future work. We can discuss this by conference call or during your visit that you were planning. We almost have Keratinosense cells, gone through the hurdles and hope we can receive it in couple of weeks.

We do appreciate continuous support from OCAC and hope to continue improving collaboration in future.

IK

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#); [AMAR GOPAL CHITTIBOYINA](#)
Cc: [Welch, Cara](#)
Subject: Re: Cosmetic project
Date: Tuesday, October 25, 2016 5:00:01 PM
Importance: High

Dear Nakisha

I am know you are busy, would you have time to meet on Thursday for lunch or after lunch. If Not, Amar is available tomorrow anytime. I am occupied but he can talk about the work if you are available.

Thanks

IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Monday, October 17, 2016 at 4:16 PM
To: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Cc: Ikhlas Khan <ikhlan@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>
Subject: RE: Cosmetic project

thank you very much for your promptness. I look forward to going through the slides and the narratives.

Regards,
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Director, Cosmetics Division
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Sent: Monday, October 17, 2016 3:35 PM
To: Sadrieh, Nakissa
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Thank you,

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Regards,

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [Katz, Linda](#); [Welch, Cara](#); [AMAR GOPAL CHITTIBOYINA](#); [Vukmanovic, Stanislav](#); [Verma, Rajeshwar *](#)
Subject: Re: Cosmetic project
Date: Wednesday, August 24, 2016 11:35:03 PM

Dear Nakissa

The purpose of this ppt was exactly what you are asking. We collected all the topics from past and what we should do in future. We will prepare all what you asked and we will submit reports as you suggested.

Keratinosens agreement has been approved by the university after a long struggle but cells should be here soon.

We will be in touch soon

ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, August 24, 2016 at 3:42 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: "Katz, Linda" <Linda.Katz@fda.hhs.gov>, Cara Welch <Cara.Welch@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>, "Verma, Rajeshwar *" <Rajeshwar.Verma@fda.hhs.gov>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Subject: RE: Cosmetic project

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- <!--[if !supportLists]-->1. <!--[endif]-->We would like to see your deliverable in the form of a report, rather than a Powerpoint presentation. This means that the report would contain the usual contents, such as introduction, materials and methods, results and a discussion and conclusion, highlighting next steps. We are asking for a report because we do not see clear questions and goals for many of the projects in the presentation. We are also missing important methodological details. Therefore, we would like to request a detailed report, written in a word format.
- <!--[if !supportLists]-->2. <!--[endif]-->Currently our interest is focused on the skin sensitization-related projects. Therefore, please prepare a separate report for the botanicals work that has been completed, and a separate report on the ongoing and planned skin sensitization projects.
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Cc: [Welch, Cara](#)
Subject: Re: Cosmetic project
Date: Tuesday, October 25, 2016 5:22:29 PM

Thanks for quick reply. According to agenda lunch time is 12:15 to 1:30. We can meet in the cafeteria during that time.

IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Tuesday, October 25, 2016 at 4:02 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Cc: Cara Welch <Cara.Welch@fda.hhs.gov>
Subject: RE: Cosmetic project

Hello,

I am at a conference tomorrow, so Thursday would be better for me. I can meet you for lunch, but I have to be back for a 1 pm meeting. when would you be available for lunch? Otherwise I can meet after 1:30 pm. Please let me know if I need to move my 1 pm meeting to a bit later. I could do that.

Regards,

Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
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Importance: High
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Cc: Katz, Linda; Welch, Cara; Vukmanovic, Stanislav; Verma, Rajeshwar *

Subject: RE: Cosmetic project

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Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: Draft Agenda
Date: Wednesday, February 17, 2016 2:03:11 PM

Thanks
Ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, February 17, 2016 at 11:48 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Subject: RE: Draft Agenda

Ikhlas,
Sorry for not responding sooner, but yes, this agenda looks fine to me. I will try to put my slides together over the next few weeks. Thanks.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Thursday, February 11, 2016 6:20 PM
To: icsb@olemiss.edu
Subject: Draft Agenda

Please review this draft agenda. Some session title, chairs and additional information needs to be fixed.
Your input will be appreciated
ik

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: Draft NCNPR_COE PMR_v4
Date: Friday, December 09, 2016 9:36:50 AM

Thanks
ik

From: "Sadrieh, Nakissa"
Date: Friday, December 9, 2016 at 6:14 AM
To: ikhlas
Subject: Fwd: Draft NCNPR_COE PMR_v4
Here are my edits. Thanks.

From: Sadrieh, Nakissa
Date: December 8, 2016 at 3:58:38 PM EST
To: Welch, Cara
Cc: Sadrieh, Nakissa
Subject: Draft NCNPR_COE PMR_v4

Cara,

I provided some language here for the PMR section on cosmetics. please feel free to make changes, as the COR, and forward to U Miss. Thank you.

From: [Amar Chittiboyina](#)
To: [Sadrieh, Nakissa](#)
Cc: [Ikhlas A. Khan](#)
Subject: RE: Draft NCNPR_COE PMR_v4
Date: Friday, December 09, 2016 11:24:44 AM
Attachments: [Safety of botanical ingredients in personal care products and cosmetics.pdf](#)

Hi Nakissa,

Thank you for the quick response. Made the suggested corrections. Attached review article on 'safety of botanical ingredients in cosmetics' covers the aspects related to hypersensitivity, irritation, phototoxicity with examples of botanicals. -Amar

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Friday, December 09, 2016 8:11 AM
To: AMAR GOPAL CHITTIBOYINA
Subject: FW: Draft NCNPR_COE PMR_v4

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, December 9, 2016 at 6:14 AM
To: ikhlas <ikhlan@olemiss.edu>
Subject: Fwd: Draft NCNPR_COE PMR_v4

Here are my edits. Thanks.

From: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Date: December 8, 2016 at 3:58:38 PM EST
To: Welch, Cara <Cara.Welch@fda.hhs.gov>
Cc: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Subject: Draft NCNPR_COE PMR_v4

Cara,

I provided some language here for the PMR section on cosmetics. please feel free to make changes, as the COR, and forward to U Miss. Thank you.

From: [Ikhlas Khan](#)
To: [Katz, Linda](#)
Cc: [Sadrieh, Nakissa](#); [Milstein, Stanley R](#); [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: ICSB conference
Date: Monday, September 28, 2015 2:27:23 PM

Hi Linda

Good to hear that. Sorry, we will miss you. I will be happy to work with Nakissa and I hope she does not have any conflict.

Nakissa, do you time this week to talk about it.

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Monday, September 28, 2015 at 12:15 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: RE: ICSB conference

Ikhlas,

We definitely have an interest in continuing this effort. Unfortunately the dates don't work for me this year, but Nakissa is willing to help with the efforts and speak if so desired. Let me know if this works for you.

Linda

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Monday, September 28, 2015 11:33 AM
To: Katz, Linda
Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA
Subject: ICSB conference

Dear Linda

Hope everything is fine. Last year we had first time session on cosmetics which Pat organized. We discuss during your visit a possibility to continue this effort. If you all think there is a merit in it please let me know and help us inviting the right people for that particular session on cosmetics.

Conference will be held at Oxford Mississippi, April 11-14, 2016.

www.oxfordicsb.org

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Friday, May 29, 2015 at 2:17 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: Thanks

Ikhlas,

Thank you as well. The time spent was both informative and useful. I look forward to our future collaboration.

Linda

Linda M. Katz, M.D., M.P.H.

Director, Office of Cosmetics and Colors

Acting Chief Medical Officer Center for Food Safety and Applied Nutrition

Food and Drug Administration
5100 Paint Branch Parkway, HFS-100
College Park, Maryland 20740

240-402-1130 (phone)
301-436-2976 (fax)

linda.katz@fda.hhs.gov

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]

Sent: Friday, May 29, 2015 3:04 PM

To: Katz, Linda

Subject: Thanks

Dear Linda

I would like to thank you for taking time to visit us. I hope you were able to get some information about the program and how we are approaching it. Please feel free to contact us if you need further information. Looking forward to continuing our collaboration.

IK

Ikhlas A. Khan, Ph.D, D. Litt (Hon. Causa)

Asst. Director, NCNPR

Director , FDA Center of Excellence

Director Center for Research in Indian Systems of Medicine (CRISM)

Director of Sino-US TCM Research Center

Research Professor Professor, Dept. of Pharmacognosy

National Center for Natural Products Research School of

Pharmacy University of Mississippi University, MS 38677

USA Tel 662/915/7821 fax 662/915/7989

<http://www.pharmacy.olemiss.edu/ncnpr/index.html>

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [Katz, Linda](#); [Milstein, Stanley R](#); [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: ICSB conference
Date: Monday, September 28, 2015 7:08:52 PM

I will be traveling on Friday. What about early next week
Ik

Sent from my iPhone

On Sep 28, 2015, at 5:06 PM, Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov> wrote:

Hello Ikhlas,
Will sometime on Friday afternoon work for you, in order to have a discussion on this topic? Thanks.
Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Monday, September 28, 2015 2:27 PM
To: Katz, Linda
Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA
Subject: Re: ICSB conference
Hi Linda
Good to hear that. Sorry, we will miss you. I will be happy to work with Nakissa and I hope she does not have any conflict.
Nakissa, do you have time this week to talk about it.
IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Monday, September 28, 2015 at 12:15 PM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: RE: ICSB conference
Ikhlas,
We definitely have an interest in continuing this effort. Unfortunately the dates don't work for me this year, but Nakissa is willing to help with the efforts and speak if so desired. Let me know if this works for you.
Linda

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Monday, September 28, 2015 11:33 AM

To: Katz, Linda
Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA
Subject: ICSB conference

Dear Linda

Hope everything is fine. Last year we had first time session on cosmetics which Pat organized. We discuss during your visit a possibility to continue this effort. If you all think there is a merit in it please let me know and help us inviting the right people for that particular session on cosmetics.

Conference will be held at Oxford Mississippi, April 11-14, 2016.

www.oxfordicsb.org

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Friday, May 29, 2015 at 2:17 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Subject: RE: Thanks

Ikhlas,

Thank you as well. The time spent was both informative and useful. I look forward to our future collaboration.

Linda

Linda M. Katz, M.D., M.P.H.

Director, Office of Cosmetics and Colors

Acting Chief Medical Officer Center for Food Safety and Applied Nutrition

Food and Drug Administration

5100 Paint Branch Parkway, HFS-100

College Park, Maryland 20740

240-402-1130 (phone)

301-436-2976 (fax)

linda.katz@fda.hhs.gov

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Friday, May 29, 2015 3:04 PM

To: Katz, Linda

Subject: Thanks

Dear Linda

I would like to thank you for taking time to visit us. I hope you were able to get some information about the program and how we are approaching it. Please feel free to contact us if you need further information. Looking forward to continuing our collaboration.

IK

Ikhlas A. Khan, Ph.D, D. Litt (Hon. Causa)

Asst. Director, NCNPR

Director , FDA Center of Excellence

Director Center for Research in Indian Systems of Medicine (CRISM)

Director of Sino-US TCM Research Center

Research Professor Professor, Dept. of Pharmacognosy

National Center for Natural Products Research School of

Pharmacy University of Mississippi University, MS 38677

USA Tel 662/915/7821 fax 662/915/7989

<http://www.pharmacy.olemiss.edu/ncnpr/index.html>

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: ICSB
Date: Monday, January 25, 2016 9:33:59 PM

Yes, you are right

Should I use "FDA perspective for the regulation of cosmetic products" as a title?

ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Monday, January 25, 2016 at 7:54 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Subject: Re: ICSB

Thanks Ikhlas. I remember that we talked about my presentation focusing on the FDA perspective for the regulation of cosmetic products. Is this what you remember too?

From: Ikhlas Khan <ikhlan@olemiss.edu>

Date: January 25, 2016 at 5:25:26 PM EST

To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>

Subject: ICSB

Dear Nakissa

Hope you are doing fine and safe in this weather. Please send me the title for your talk. We were able to get David Moyler from Britain as speaker

Here is the list of speakers for your session.

Moderator and Session Chair:

1:00-1:30 **Nakissa Sadrich**, Director Cosmetic Division, FDA

1:30-2:00 **Cindy Angerhofer**, Executive Director of Botanical Research, Aveda,
"Ayurvedic Herbs as Sources of Cosmetic Ingredients"

2:00- 2:30 **Amar Chittiboyina**, Senior Research Scientist, University of Mississippi,
"Alternative Testing Methods For Estimating The Skin Sensitization Potential Of Natural Products"

2:30-3:00 **David Moyler**, Regulatory Consultant, IFRA, IOFI, RIFM and the British Essential Oils Association, *"NCS; Global UN-GHS regulations implementation", which seems to me to fit the brief"*

I have not finalized the time and date yet

Thanks

IK

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: ICSB
Date: Wednesday, November 25, 2015 11:15:42 AM

Thanks
IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, November 25, 2015 at 10:06 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Re: ICSB

Yes, I was planning on doing this. Thanks.

From: Ikhlas Khan <ikhlan@olemiss.edu>
Date: November 25, 2015 at 10:19:04 AM EST
To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Cc: Katz, Linda <Linda.Katz@fda.hhs.gov>
Subject: ICSB

Dear Nakissa

Linda is working on getting speaker from Europe but I was thinking that you should also give an overview of US regulatory status and efforts being done by OCAC.

Please let me know if you are amenable to this idea

IK

Happy Thanks Giving

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: ICSB
Date: Wednesday, October 07, 2015 2:28:09 PM

Ok.
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, October 7, 2015 at 11:58 AM
To: Ikhlas Khan <ikh@olemiss.edu>
Subject: Re: ICSB

So let's do tomorrow after 4 pm. Thanks.

Sent from my BlackBerry 10 smartphone.

From: Ikhlas Khan
Sent: Wednesday, October 7, 2015 12:52 PM
To: Sadrieh, Nakissa
Subject: Re: ICSB

After 4 will be fine. tomorrow afternoon should work too.
Office# 662 915 7821
Cell# (b) (6)
IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, October 7, 2015 at 10:22 AM
To: Ikhlas Khan <ikh@olemiss.edu>
Subject: Re: ICSB

Sorry for not responding. Maybe after 4 pm today or tomorrow afternoon?

Sent from my BlackBerry 10 smartphone.

From: Ikhlas Khan
Sent: Wednesday, October 7, 2015 11:07 AM
To: Sadrieh, Nakissa
Subject: ICSB

Dear Nakissa
I was waiting for your response but looks like email never reached you. Do you have sometime this week to discuss about the session.
It should not take long but it will get us started.
Thanks
IK

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: ICSB
Date: Wednesday, October 07, 2015 12:52:37 PM

After 4 will be fine. tomorrow afternoon should work too.

Office# 662 915 7821

Cell# (b) (6)

IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Wednesday, October 7, 2015 at 10:22 AM

To: Ikhlas Khan <khan@olemiss.edu>

Subject: Re: ICSB

Sorry for not responding. Maybe after 4 pm today or tomorrow afternoon?

Sent from my BlackBerry 10 smartphone.

From: Ikhlas Khan
Sent: Wednesday, October 7, 2015 11:07 AM
To: Sadrieh, Nakissa
Subject: ICSB

Dear Nakissa

I was waiting for your response but looks like email never reached you. Do you have sometime this week to discuss about the session.

It should not take long but it will get us started.

Thanks

IK

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: icsb@olemiss.edu
Subject: Re: ICSB
Date: Monday, March 06, 2017 8:59:35 PM

Hi Nakissa

I am sorry to hear that. It will be big loss, not having you here but I understand that working in a uncertain environment is always challenging. Yes, we will try to keep you posted with the research here and you should always feel free to call or email if you need any information from us. It took some time but things are getting in shape and hopefully it will be developed in an impactful program.

Going to Miss you. Wish you the best

IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Monday, March 6, 2017 at 7:52 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: Ikhlas Khan <ICSB@olemiss.edu>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Subject: Re: ICSB

Ikhlas,

We are currently going through a very busy period related to a number of simultaneous priority regulatory activities, while navigating a fragile transition period in the government. Consequently, I won't be able to come to U Miss this year, and I won't be able to give a presentation. I am sorry for the inconvenience that this creates, and I hope to be able to come next year.

In the meantime, we hope to continue having regular teleconferences to talk about the research that your lab is doing for us. The work that your lab is very important to us, and I hope that we can get some good results that I will be able to showcase next year at the conference.

Thanks and sorry about this year and the late notification. The federal government is a busy and somewhat interesting place right now.

Nakissa.

From: Ikhlas Khan <ikhlan@olemiss.edu>
Date: March 6, 2017 at 11:15:27 AM EST
To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Cc: icsb@olemiss.edu <icsb@olemiss.edu>
Subject: ICSB

Hi Nakissa

I know you are busy but we need your abstract and title to complete the booklet. I will appreciate if you can forward it to us.

Appreciate it

ik

From: [Ikhlas Khan](#)
To: [Vukmanovic, Stanislav](#); [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#); [Herrmann, Michelle](#); [SHABANA I KHAN](#)
Subject: Re: keratinosens
Date: Wednesday, April 06, 2016 5:06:00 PM

Dear Nakissa and Stan

After making several rounds of conference call with different departments at University and company in Switzerland, its clear that company won't change their policy and University can't accept their conditions.

Nut after talking to our Lawyer at the university, he suggested some other mechanism which probably will work. It means we can have cell lines in near future.
I will keep you posted.

IK

P.S: Nakissa, I assume you all set for the trip, here is my cell# (b) (6)

From: Ikhlas Khan <ikhlan@olemiss.edu>
Date: Wednesday, February 17, 2016 at 1:54 PM
To: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>, shabana <skhan@olemiss.edu>
Subject: Re: keratinosens

Dear Stan

Just wanted to give update about the cell line. As you can imagine, we are still working through the university system. Going back and forth with the agreement.
Hope I will have some good news soon
IK

From: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Date: Monday, January 25, 2016 at 12:30 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>, shabana <skhan@olemiss.edu>
Subject: RE: keratinosens

Great! Thank you so much.

Best,
Stan

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Monday, January 25, 2016 1:26 PM
To: Vukmanovic, Stanislav; Sadrieh, Nakissa

Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle; SHABANA I KHAN

Subject: Re: keratinosens

Hi Stan

We had a day off last Friday but it was not too bad. We had back and forth email exchange with the company and they send us transfer agreement to fill. Last week we send them that. We are waiting to hear from them. Once approved, they will send us invoice and we have to deal with our purchasing department.

we will keep you posted

IK

From: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>

Date: Monday, January 25, 2016 at 12:13 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle"

<Michelle.Herrmann@fda.hhs.gov>

Subject: RE: keratinosens

Hi Ikhlas,

I hope you had nice holidays and not as much snowstorm as we had few days ago.

I just wanted to touch base and see whether by now you may have an idea how easy (or difficult) will it be for you to acquire the license for Keratinocyte™ assay.

Please, let us know any updates you may have.

Best,

Stan

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Friday, December 11, 2015 10:56 AM

To: Sadrieh, Nakissa; Vukmanovic, Stanislav

Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle

Subject: Re: keratinosens

Dear Nakissa

We can call you at your office# or you can give a number to call. I assume it's 2:00 CST it's 2 EST your time I will request to delay it to 3:EST or 2:30 EST

ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Friday, December 11, 2015 at 9:52 AM

To: Ikhlas Khan <ikhlan@olemiss.edu>, "Vukmanovic, Stanislav"

<Stanislav.Vukmanovic@fda.hhs.gov>

Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle"

<Michelle.Herrmann@fda.hhs.gov>

Subject: RE: keratinosens

Ikhlas, are we still talking at 2 pm today? What telephone number would you like us to call, or are you calling us? Thanks.

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Thursday, December 10, 2015 12:00 PM
To: Vukmanovic, Stanislav; Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle
Subject: Re: keratinosens
Thanks, yes it will be helpful to us too
ik

From: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Date: Thursday, December 10, 2015 at 10:45 AM
To: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, Ikhlas Khan <ikhan@olemiss.edu>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>
Subject: RE: keratinosens
Dear Ikhlas and Amar,
In order to have a more productive discussion tomorrow, it may be a good idea to share with you the preliminary plan of the project that we would like to be performed. That way the discussion can be more informative. Your input in design is welcome.
Best,
Stan

From: Sadrieh, Nakissa
Sent: Thursday, December 10, 2015 11:01 AM
To: Ikhlas Khan
Cc: Vukmanovic, Stanislav; AMAR GOPAL CHITTIBOYINA
Subject: Re: keratinosens
Tomorrow is better for me. Maybe we can talk sometime after 2 pm? I will check with my calendar and confirm. Thanks.

From: Ikhlas Khan <ikhan@olemiss.edu>
Date: December 10, 2015 at 10:31:28 AM EST
To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Cc: Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: Re: keratinosens
Importance: High
Dear Nakissa
I am back in office and had discussion with Amar and others. Please let me know when you have time for discussion. We are available this afternoon and also tomorrow. If it does not work for you, please suggest for next week
Thanks
IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, December 2, 2015 at 8:20 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>

Subject: Re: keratinosens

Thank you Ikhlas. Once I hear from you, we can discuss our proposed study.

From: Ikhlas Khan <ikhlan@olemiss.edu>

Date: December 2, 2015 at 9:18:38 PM EST

To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>

Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>

Subject: Re: keratinosens

Dear Nakissa

We did gather information and tried keratinosens assay but than we were told to focus on H-Clat. We have H-Clat right now that we are using for known compounds but we will be happy to bring Keratinosens assay on board.

I will check the status and let you know.

IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Wednesday, December 2, 2015 at 5:13 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>

Subject: keratinosens

Hi Ikhlas,

I hope that you are well. I am writing to ask you whether U Miss has the Keratinosens assay up and running? This is one of the assays that we have an interest in, and in past discussions, I think that U Miss had mentioned that the assay was available for testing cosmetic ingredients. as you know, Stan Vukmanovic on my staff is working on the immunogenicity of fragrance allergens, and we are hoping to have a battery of tests set up both here and at U Miss, that would allow us to assess the sensitization potential of cosmetic ingredients. I have cc'ed Stan on this email, so that he may be involved in our conversation.

Please let me know if you are in a position to use the Keratinosens assay, because we are interested in a study design that we have developed, to test some fragrance allergens. Thank you.

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Vukmanovic, Stanislav](#); [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#); [Herrmann, Michelle](#); [SHABANA I KHAN](#)
Subject: Re: keratinosens
Date: Thursday, February 18, 2016 11:48:58 AM

Dear Stan

Thanks for asking our input. We completely agree with your approach. As we mentioned in last call we are planning to initiate h-Clat and it will be easy to add DPRA since no agreement is required. Hopefully we have all three assay up and running soon and start screening these known 26 compounds first.

Please let us know if you need further information from us in the meantime we will look in to DPRA assay.

Thanks

IK

From: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Date: Wednesday, February 17, 2016 at 1:29 PM
To: Ikhlas Khan <ikhan@olemiss.edu>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>, shabana <skhan@olemiss.edu>
Subject: RE: keratinosens

Thank you so much for your update. I can imagine that there may be a number of hurdles one has to jump over to get this license. Patience is a virtue...

While we are waiting, there is another project that would like to initiate. Part of this project, too, will require Keratinosens license, but the other parts should be doable. In a nutshell, we would like to start generating independent in vitro data on allergens using validated methods (DPRA, Keratinosens, and h-CLAT). We would like to start with the 26 fragrances (hence the attached proposal talks only about these compounds), but the project would eventually expand to other potential allergens. Could you please have a look at the attached proposal and let us know your thoughts.

Thanks again for all your efforts.

Best,

Stan

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Wednesday, February 17, 2016 1:55 PM
To: Vukmanovic, Stanislav; Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle; SHABANA I KHAN
Subject: Re: keratinosens
Dear Stan

Just wanted to give update about the cell line. As you can imagine, we are still working through the university system. Going back and forth with the agreement. Hope I will have some good news soon

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From: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Date: Monday, January 25, 2016 at 12:30 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>, shabana <skhan@olemiss.edu>

Subject: RE: keratinosens

Great! Thank you so much.

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To: Vukmanovic, Stanislav; Sadrieh, Nakissa

Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle; SHABANA I KHAN

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Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>

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Food and Drug Administration (FDA)
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Cc: [AMAR GOPAL CHITTIBOYINA](#); [Herrmann, Michelle](#); [SHABANA I KHAN](#)
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Importance: High

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I am back in office and had discussion with Amar and others. Please let me know when you have time for discussion. We are available this afternoon and also tomorrow. If it does not work for you, please suggest for next week

Thanks

IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, December 2, 2015 at 8:20 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: Re: keratinosens

Thank you Ikhlas. Once I hear from you, we can discuss our proposed study.

From: Ikhlas Khan <ikhlan@olemiss.edu>
Date: December 2, 2015 at 9:18:38 PM EST
To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>
Subject: Re: keratinosens

Dear Nakissa

We did gather information and tried keratinosens assay but than we were told to focus on H-Clat. We have H-Clat right now that we are using for known compounds but we will be happy to bring Keratinosens assay on board.

I will check the status and let you know.

IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Wednesday, December 2, 2015 at 5:13 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>

Subject: keratinosens

Hi Ikhlas,

I hope that you are well. I am writing to ask you whether U Miss has the Keratinosens assay up and running? This is one of the assays that we have an interest in, and in past discussions, I think that U Miss had mentioned that the assay was available for testing cosmetic ingredients. as you know, Stan Vukmanovic on my staff is working on the immunogenicity of fragrance allergens, and we are hoping to have a battery of tests set up both here and at U Miss, that would allow us to assess the sensitization potential of cosmetic ingredients. I have cc'ed Stan on this email, so that he may be involved in our conversation.

Please let me know if you are in a position to use the Keratinosens assay, because we are interested in a study design that we have developed, to test some fragrance allergens. Thank you.

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Vukmanovic, Stanislav](#); [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#); [Herrmann, Michelle](#)
Subject: Re: keratinosens
Date: Thursday, December 10, 2015 12:00:39 PM

Thanks, yes it will be helpful to us too
ik

From: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Date: Thursday, December 10, 2015 at 10:45 AM
To: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, Ikhlas Khan <ikhlan@olemiss.edu>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>
Subject: RE: keratinosens

Dear Ikhlas and Amar,

In order to have a more productive discussion tomorrow, it may be a good idea to share with you the preliminary plan of the project that we would like to be performed. That way the discussion can be more informative. Your input in design is welcome.

Best,
Stan

From: Sadrieh, Nakissa
Sent: Thursday, December 10, 2015 11:01 AM
To: Ikhlas Khan
Cc: Vukmanovic, Stanislav; AMAR GOPAL CHITTIBOYINA
Subject: Re: keratinosens

Tomorrow is better for me. Maybe we can talk sometime after 2 pm? I will check with my calendar and confirm. Thanks.

From: Ikhlas Khan <ikhlan@olemiss.edu>
Date: December 10, 2015 at 10:31:28 AM EST
To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Cc: Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: Re: keratinosens

Importance: High

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Date: December 2, 2015 at 9:18:38 PM EST

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Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>

Subject: Re: keratinosens

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Date: Wednesday, December 2, 2015 at 5:13 PM

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Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>

Subject: keratinosens

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Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [Vukmanovic, Stanislav](#); [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: keratinosens
Date: Thursday, December 10, 2015 10:31:28 AM
Importance: High

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Cc: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: Re: keratinosens

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To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Vukmanovic, Stanislav"

<Stanislav.Vukmanovic@fda.hhs.gov>

Subject: keratinosens

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4300 River Road

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College Park, MD 20740

Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [Vukmanovic, Stanislav](#); [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: keratinosens
Date: Wednesday, December 02, 2015 9:18:38 PM

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From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, December 2, 2015 at 5:13 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Subject: keratinosens

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4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: keratinosens
Date: Wednesday, April 06, 2016 5:37:00 PM

Thanks, Talk is 30 min including Q&A. Yes we can upload but still safe to bring flash drive

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, April 6, 2016 at 4:20 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: keratinosens

Thanks Ikhlas. We can chat next week about this.

I am all set from my trip, I think. I will send you my slides by Friday at the latest. You will be able to have them uploaded for the presentation? How long is my talk? thanks.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Wednesday, April 06, 2016 5:06 PM
To: Vukmanovic, Stanislav; Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle; SHABANA I KHAN
Subject: Re: keratinosens

Dear Nakissa and Stan

After making several rounds of conference call with different departments at University and company in Switzerland, its clear that company won't change their policy and University can't accept their conditions.

Nut after talking to our Lawyer at the university, he suggested some other mechanism which probably will work. It means we can have cell lines in near future.

I will keep you posted.

IK

P.S: Nakissa, I assume you all set for the trip, here is my cell# (b) (6)

From: Ikhlas Khan <ikhlan@olemiss.edu>
Date: Wednesday, February 17, 2016 at 1:54 PM
To: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>, shabana <skhan@olemiss.edu>
Subject: Re: keratinosens
Dear Stan

Just wanted to give update about the cell line. As you can imagine, we are still working through the university system. Going back and forth with the agreement. Hope I will have some good news soon
IK

From: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Date: Monday, January 25, 2016 at 12:30 PM
To: Ikhlas Khan <ikhan@olemiss.edu>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>, shabana <skhan@olemiss.edu>
Subject: RE: keratinosens
Great! Thank you so much.
Best,
Stan

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Monday, January 25, 2016 1:26 PM
To: Vukmanovic, Stanislav; Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle; SHABANA I KHAN
Subject: Re: keratinosens
Hi Stan
We had a day off last Friday but it was not too bad. We had back and forth email exchange with the company and they send us transfer agreement to fill. Last week we send them that. We are waiting to hear from them. Once approved, they will send us invoice and we have to deal with our purchasing department.
we will keep you posted
IK

From: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>
Date: Monday, January 25, 2016 at 12:13 PM
To: Ikhlas Khan <ikhan@olemiss.edu>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle" <Michelle.Herrmann@fda.hhs.gov>
Subject: RE: keratinosens
Hi Ikhlas,
I hope you had nice holidays and not as much snowstorm as we had few days ago.
I just wanted to touch base and see whether by now you may have an idea how easy (or difficult) will it be for you to acquire the license for Keratinocyte™ assay.
Please, let us know any updates you may have.
Best,
Stan

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Friday, December 11, 2015 10:56 AM
To: Sadrieh, Nakissa; Vukmanovic, Stanislav
Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle
Subject: Re: keratinosens
Dear Nakissa
We can call you at your office# or you can give a number to call. I assume it's 2:00 CST it's 2 EST your time I will request to delay it to 3:EST or 2:30 EST
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Friday, December 11, 2015 at 9:52 AM

To: Ikhlas Khan <ikhlan@olemiss.edu>, "Vukmanovic, Stanislav"

<Stanislav.Vukmanovic@fda.hhs.gov>

Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle"

<Michelle.Herrmann@fda.hhs.gov>

Subject: RE: keratinosens

Ikhlas, are we still talking at 2 pm today? What telephone number would you like us to call, or are you calling us? Thanks.

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Thursday, December 10, 2015 12:00 PM

To: Vukmanovic, Stanislav; Sadrieh, Nakissa

Cc: AMAR GOPAL CHITTIBOYINA; Herrmann, Michelle

Subject: Re: keratinosens

Thanks, yes it will be helpful to us too

ik

From: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>

Date: Thursday, December 10, 2015 at 10:45 AM

To: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, Ikhlas Khan <ikhlan@olemiss.edu>

Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, "Herrmann, Michelle"

<Michelle.Herrmann@fda.hhs.gov>

Subject: RE: keratinosens

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Cc: "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>

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To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Vukmanovic, Stanislav" <Stanislav.Vukmanovic@fda.hhs.gov>

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Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [Jennifer S. Taylor](#)
Subject: Re: list
Date: Thursday, July 23, 2015 11:01:14 AM

Thanks.
IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Thursday, July 23, 2015 at 9:57 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: ac <jnnfrtyl@olemiss.edu>
Subject: RE: list

The meeting is scheduled from 10:30 to 12:30 at this point. So it is safe to book your tickets for the afternoon. I suggest not too much before 5 pm departure, to give you time for lunch, to get to the airport and go through security.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Thursday, July 23, 2015 9:58 AM
To: Sadrieh, Nakissa
Cc: Jennifer S. Taylor
Subject: Re: list

Dear Nakissa

Its safe to book the ticket in the 13th afternoon and meeting will take place in the morning
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, July 17, 2015 at 11:44 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: RE: list

We shall try to schedule the meeting for around 10:30 am.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)

4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Friday, July 17, 2015 12:42 PM

To: Sadrieh, Nakissa

Subject: Re: list

Prefer morning so we wont be rushed to catch evening flight

Sent from my iPhone

On Jul 17, 2015, at 10:58 AM, Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov> wrote:

We are trying to schedule the meeting between 2 and 4 pm. Is that OK with you or do you prefer from 10 am to 12pm?

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Friday, July 17, 2015 11:37 AM

To: Sadrieh, Nakissa; Katz, Linda

Cc: Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Larry Walker

Subject: Re: list

Hi Nakissa

Good, My presentation should cover all the topics yo suggested and after an overview we can discuss future plan.

We will plan to book return flight on 13th evening so we have enough time.

ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Friday, July 17, 2015 at 10:20 AM

To: Ikhlas Khan <ikhlan@olemiss.edu>, "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Cc: "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>, Larry Walker <walker@olemiss.edu>

Subject: RE: list

Ikhlas,

It would be nice to get an overview that focuses on the work that the University of Mississippi has done to date on 1) botanicals, and specifically the arbutins, and 2) in vitro and in chemico methods that are available at U Miss, for screening of cosmetic sensitizers. I would like perhaps some slides included on potential areas of future work, based on our interests, which are covered by the 2 points indicated above. Please keep in mind that any work is to be directly linked to a regulatory outcome, and this is where I would probably provide guidance. Thanks.

Regards,

Nakissa Sadrieh, Ph.D.

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College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Friday, July 17, 2015 11:05 AM

To: Katz, Linda

Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Larry Walker

Subject: Re: list

Dear Linda

Thanks for quick response. Our meeting will end on 12th evening, we can stay next day and have meeting on 13th and return in the afternoon or evening back.

We have provided ppts but if you think it will be useful to give an overview, I can present the same presentation which I gave during your visit.

Nakissa, please let me know if you have some thoughts about the agenda or you would like to cover any specific topic

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Date: Friday, July 17, 2015 at 7:24 AM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>

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Linda

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Thursday, July 16, 2015 12:15 PM

To: Katz, Linda

Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO

Subject: Re: list

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To: Katz, Linda

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Subject: Re: list

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Farnesol*

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Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)

Isoeugenol*

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Linalool* (oxidised)

*including their respective esters

The 2012 SCCS Opinion also recommends that fragrance pre-haptens and pro-haptens of several terpene fragrance materials should be considered as putative “allergens” and regulated in the same way as the allergens by the EC (Among them are limonene, linalool, linalyl acetate, geraniol, geranial, α-terpinene, eugenol, isoeugenol, and cinnamyl alcohol).

Let me know if you need any further information at this time regarding allergens.

On a different note, let me know if you have a report and list with description of in vitro assays, including validation, that actually describes the methods that you have developed for arbutin.

Linda

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Sent: Wednesday, June 17, 2015 1:21 PM

To: Ikhlas Khan

Cc: Katz, Linda

Subject: RE: list

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By copy of this note, I'll alert Linda to your request for the fragrance allergen list. We talked about it during our visit and she may have assigned it to someone already, but I'm not sure.

Is there a conference call already scheduled, perhaps with the dietary supplement group and others or are you talking about something else?

Hope all is well.

PH

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]

Sent: Friday, June 05, 2015 10:58 AM

To: Hansen, Patricia A

Subject: list

Hi Pat

I know this is your first week and must be busy. Could you please share the list of 26 and may be 80 if possible.

It wil help to us to see what is coming but you will still have time to prioritize before we have conference call

Thanks

IK

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [Jennifer S. Taylor](#)
Subject: Re: list
Date: Thursday, July 23, 2015 9:57:36 AM

Dear Nakissa

Its safe to book the ticket in the 13th afternoon and meeting will take place in the morning
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, July 17, 2015 at 11:44 AM
To: Ikhlas Khan <ikhan@olemiss.edu>
Subject: RE: list

We shall try to schedule the meeting for around 10:30 am.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Friday, July 17, 2015 12:42 PM
To: Sadrieh, Nakissa
Subject: Re: list
Prefer morning so we wont be rushed to catch evening flight

Sent from my iPhone

On Jul 17, 2015, at 10:58 AM, Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov> wrote:

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To: Sadrieh, Nakissa; Katz, Linda

Cc: Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Larry Walker

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Sent: Friday, July 17, 2015 11:05 AM

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From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Monday, June 29, 2015 1:04 PM

To: Katz, Linda

Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO

Subject: Re: list

Dear Linda

We will send the information soon.

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Monday, June 29, 2015 at 10:48 AM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>

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Cc: Hansen, Patricia A; Sadrieh, Nakissa; Milstein, Stanley R

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Date: Thursday, June 25, 2015 at 9:47 AM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "patricia.hansen@fda.hhs.gov" <patricia.hansen@fda.hhs.gov>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>

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To: Hansen, Patricia A
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From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: list
Date: Friday, July 17, 2015 12:42:22 PM

Prefer morning so we wont be rushed to catch evening flight

Sent from my iPhone

On Jul 17, 2015, at 10:58 AM, Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov> wrote:

We are trying to schedule the meeting between 2 and 4 pm. Is that OK with you or do you prefer from 10 am to 12pm?

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]

Sent: Friday, July 17, 2015 11:37 AM

To: Sadrieh, Nakissa; Katz, Linda

Cc: Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Larry Walker

Subject: Re: list

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CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO

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Tel: 240-402-2194

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Date: Friday, July 17, 2015 at 7:24 AM
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Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>
Subject: RE: list

Ikhlas,

I will be out of the office when you are here in August. However, Nakissa and Stan would love to meet with you and will probably also invite our colleagues in OARSA, who are doing related research, to attend for a broader discussion of methods and direction. Let me know your actual availability, including the amount of time you will have to spend with us so that we can make arrangements for a room. Also let me know if you will be making any formal presentations. We will work on an agenda from our end which we will share. Because I will be out of the office from August 3 through August 13, Nakissa will be your point of contact.

Linda

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Thursday, July 16, 2015 12:15 PM
To: Katz, Linda
Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: list

Dear Linda

During your visit we discussed a possibility to meet the OCAC group during our visit to CFSAN in August. We have COE meeting on August 12th. We can plan to meet on 13th if its feasible.

Let me know if we would like to meet and we will make our return travel plan accordingly.

ik

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Friday, July 10, 2015 at 10:45 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>

Subject: RE: list

Ikhlas,

Thanks so much for this information. We will be back in touch.

Have a nice weekend as well.

Linda

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]

Sent: Friday, July 10, 2015 11:10 AM

To: Katz, Linda

Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO

Subject: Re: list

Dear Linda

Attached, please find separate ppts to describe the results and approaches. Some slides might need further explanation, we can setup a call to go through over these slides if needed.

In the meantime anyone has a question, please feel free to contact us.

Have a nice weekend

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And publications

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Date: Monday, June 29, 2015 at 12:45 PM

To: Ikhlas Khan <ikhan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>

Subject: RE: list

Thanks very much!

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]

Sent: Monday, June 29, 2015 1:04 PM

To: Katz, Linda

Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO

Subject: Re: list

Dear Linda

We will send the information soon.

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Date: Monday, June 29, 2015 at 10:48 AM

To: Ikhlas Khan <ikhan@olemiss.edu>

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Please provide a summary of the in vitro sensitization assays available and the ingredients

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To: Katz, Linda

Cc: Hansen, Patricia A; Sadrieh, Nakissa; Milstein, Stanley R

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Dear Linda

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Thanks

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From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Thursday, June 25, 2015 at 9:47 AM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "patricia.hansen@fda.hhs.gov" <patricia.hansen@fda.hhs.gov>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>

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However, we are interested in the remainder of those listed below from Table 13-5.

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Cinnamyl Alcohol*

Citral

Coumarin

Eugenol*

Farnesol*

Geraniol*

Hydroxycitronellal

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Linalool* (oxidised)

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The 2012 SCCS Opinion also recommends that fragrance pre-haptens and pro-haptens of several terpene fragrance materials should be considered as putative "allergens" and regulated in the same way as the allergens by the EC (Among them are limonene, linalool, linalyl acetate, geraniol, geranial, α-terpinene, eugenol, isoeugenol, and cinnamyl alcohol).

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To: Ikhlas Khan
Cc: Katz, Linda
Subject: RE: list

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Hope all is well.

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From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
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To: Hansen, Patricia A
Subject: list

Hi Pat

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It will help to us to see what is coming but you will still have time to prioritize before we have conference call

Thanks

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From: [Ikhlas Khan](#)
To: [Katz, Linda](#)
Cc: [Sadrieh, Nakissa](#); [Milstein, Stanley R](#); [AMAR GOPAL CHITTIBOYINA](#); [CRISTINA AVONTO](#)
Subject: Re: list
Date: Thursday, July 16, 2015 12:14:36 PM

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Linda

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Cc: Katz, Linda
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Is there a conference call already scheduled, perhaps with the dietary supplement group and others or are you talking about something else?

Hope all is well.

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It will help to us to see what is coming but you will still have time to prioritize before we have conference call

Thanks

IK

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To: [Sadrieh, Nakissa](#); [Katz, Linda](#)
Cc: [Milstein, Stanley R](#); [AMAR GOPAL CHITTIBOYINA](#); [CRISTINA AVONTO](#)
Subject: Re: list
Date: Monday, July 13, 2015 3:56:49 PM

Dear Nakissa

Your approach sounds great. It will avoid duplication, give more in depth information by using more bioassay.

We will give you full information about the assays we have and how they will be used.
IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Monday, July 13, 2015 at 2:50 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>, "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Cc: "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>
Subject: RE: list

Thank you Ikhlas. I would very much like to a definitive list of in vitro assays that are ready to go at the University of Mississippi, for evaluating fragrance allergens. Those assays that you do not have available, we can try to set up in our labs here. I just want to make sure that we do not duplicate efforts, and that we have complementary assays both here and at the University of Mississippi, with possibly overlap of one assay, so that we can compare the results from both sites. Does this approach sound reasonable to you?

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Monday, July 13, 2015 3:20 PM
To: Sadrieh, Nakissa; Katz, Linda
Cc: Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: list

Dear Nakissa

Good to hear from you. LLNA and DPRA were used in our studies but sample were tested outside as. To screening botanicals and pure compounds, we have developed two assays which are included in the presentations we sent earlier.

H-CLAT is not used yet since its going under validation and checking the reproducibility before testing real compounds.

Cristina has given presentation at ICSB about new screens and presented Pros and Cons of these assays.

She will send the presentation to you soon.
Amar will be back next week and we will discuss about assays.
Please let us know if you have further questions.
IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Monday, July 13, 2015 at 11:59 AM
To: Ikhlas Khan <ikhan@olemiss.edu>, "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Cc: "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Subject: RE: list

Dear Dr. Khan,

Thank you for the information that you have sent us. Can I please ask for a clarification? In your slides, you mention that U Miss has the H-CLAT assay (Human Cell Line Activation Test) up and running, however, in previous briefings from Diego Rua who worked for OCAC, we were also told that the following assays had been used in work conducted for OCAC, by U Miss:

- <!--[if !supportLists]-->1. <!--[endif]-->LLNA= Local lymph node assay
- <!--[if !supportLists]-->2. <!--[endif]-->EASA = Electrophilic Allergen Screening Assay
- <!--[if !supportLists]-->3. <!--[endif]-->DPRA = Direct peptide reactivity assay

Are the above 3 assays also available at U Miss? Please provide me with a description (the basis for the assay and what is measured) of all the available in vitro sensitization assays available at U Miss, as well as the pros and cons of these assays, meaning what are their strengths and weaknesses. This will help us in deciding which of these assays we may wish to use, and for which projects. Also, please let me know of other in vitro assays that you are aware of, for sensitization assessment, that we might want to look into, even if U Miss does not currently have these assay up and running. Specifically, please look at the link below, and the list provided in Table 1, of other methods that might be useful for the assessment of cosmetic product sensitizers. Thank you.

<http://www.sciencedirect.com/science/article/pii/S0273230015001415#t0005>

Regards,
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Are the above 3 assays also available at U Miss? Please provide me with a description (the basis for the assay and what is measured) of all the available in vitro sensitization assays available at U Miss, as well as the pros and cons of these assays, meaning what are their strengths and weaknesses. This will help us in deciding which of these assays we may wish to use, and for which projects. Also, please let me know of other in vitro assays that you are aware of, for sensitization assessment, that we might want to look into, even if U Miss does not currently have these assay up and running. Specifically, please look at the link below, and the list provided in Table 1, of other methods that might be useful for the assessment of cosmetic product sensitizers. Thank you.

<http://www.sciencedirect.com/science/article/pii/S0273230015001415#t0005>

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Friday, July 10, 2015 11:10 AM

To: Katz, Linda

Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO

Subject: Re: list

Dear Linda

Attached, please find separate ppts to describe the results and approaches. Some slides might need further explanation, we can setup a call to go through over these slides if needed.

In the meantime anyone has a question, please feel free to contact us.

Have a nice weekend

IK

And publications

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Monday, June 29, 2015 at 12:45 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>

Subject: RE: list

Thanks very much!

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Monday, June 29, 2015 1:04 PM

To: Katz, Linda

Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO

Subject: Re: list

Dear Linda

We will send the information soon.

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Monday, June 29, 2015 at 10:48 AM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>

Subject: RE: list

Ikhlas,

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Please provide a summary of the in vitro sensitization assays available and the ingredients

From: [Ikhlas Khan](#)
To: [Katz, Linda](#)
Cc: [Sadrieh, Nakissa](#); [Milstein, Stanley R](#); [AMAR GOPAL CHITTIBOYINA](#); [CRISTINA AVONTO](#)
Subject: Re: list
Date: Friday, July 10, 2015 11:14:14 AM
Attachments: [inchemico methods case studies.pptx](#)
[FDA summary in vitro assays-v2.pptx](#)
[ARBUTIN summary.pptx](#)
[Arbutin stability.pdf](#)
[14123 Wang_Y.pdf](#)

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Dear Linda

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Date: Thursday, June 25, 2015 at 9:47 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: "patricia.hansen@fda.hhs.gov" <patricia.hansen@fda.hhs.gov>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>
Subject: FW: list

Ikhlas,

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Linalool* (oxidised)
*including their respective esters

The 2012 SCCS Opinion also recommends that fragrance pre-haptens and pro-haptens of several terpene fragrance materials should be considered as putative “allergens” and regulated in the same way as the allergens by the EC (Among them are limonene, linalool, linalyl acetate, geraniol, a-terpinene, eugenol, isoeugenol, and cinnamyl alcohol).

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Cc: Katz, Linda
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Hope all is well.

PH

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Sent: Friday, June 05, 2015 10:58 AM
To: Hansen, Patricia A
Subject: list

Hi Pat

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It wil help to us to see what is coming but you will still have time to prioritize before we have conference call

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To: [Katz, Linda](#)
Cc: [Sadrieh, Nakissa](#); [Milstein, Stanley R](#); [AMAR GOPAL CHITTIBOYINA](#); [CRISTINA AVONTO](#)
Subject: Re: list
Date: Monday, June 29, 2015 1:04:38 PM

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To: Ikhlas Khan <ikhlan@olemiss.edu>
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To: [Katz, Linda](#)
Cc: [Hansen, Patricia A](#); [Sadrieh, Nakissa](#); [Milstein, Stanley R](#)
Subject: Re: list
Date: Monday, June 29, 2015 10:54:56 AM

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Thanks

IK

From: [Amar Chittiboyina](#)
To: [Sadrieh, Nakissa](#); ["Ikhlas Khan"](#); [Katz, Linda](#)
Cc: [Milstein, Stanley R](#)
Subject: RE: list
Date: Monday, August 17, 2015 11:38:53 AM
Attachments: [2015_SitevisitOCAC.pdf](#)
[2015_CRT_NMR.pdf](#)
[LLna-pot_3b_appc_brd_annexii-1.pdf](#)

Dear Nakissa,

Thank you and your team for hosting us. The meeting was very productive and we are looking forward to work with you on OCAC needs on botanicals in cosmetics including 26 ingredients.

A copy of Dr. Khan's presentation (in PDF) is attached along with recently accepted publication on NMR spectroscopy.

Can you pass the following information to Raj also?

1. LLNA data on coumarin was one of the test article in the document on "Comparative LLNA, Guinea Pig, and Human Data Used in the Performance Evaluation". Please refer page #23 for coumarin data (attached).

ntp.niehs.nih.gov/iccvm/docs/immunotox_docs/llna-pot/3b-appc-brd-annexii-1.pdf

Please let us know if you need any additional information.

Sincerely

Amar

From: Sadrieh, Nakissa [mailto:Nakissa.Sadrieh@fda.hhs.gov]
Sent: Friday, July 17, 2015 10:58 AM
To: 'Ikhlas Khan'; Katz, Linda
Cc: Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Larry Walker
Subject: RE: list

We are trying to schedule the meeting between 2 and 4 pm. Is that OK with you or do you prefer from 10 am to 12pm?

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Friday, July 17, 2015 11:37 AM
To: Sadrieh, Nakissa; Katz, Linda
Cc: Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Larry Walker
Subject: Re: list

Hi Nakissa

Good, My presentation should cover all the topics yo suggested and after an overview we can discuss future plan.

We will plan to book return flight on 13th evening so we have enough time.

ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, July 17, 2015 at 10:20 AM
To: Ikhlas Khan <ikhlan@olemiss.edu>, "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Cc: "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>, Larry Walker <lwalker@olemiss.edu>
Subject: RE: list

Ikhlas,

It would be nice to get an overview that focuses on the work that the University of Mississippi has done to date on 1) botanicals, and specifically the arbutins, and 2) in vitro and in chemico methods that are available at U Miss, for screening of cosmetic sensitizers. I would like perhaps some slides included on potential areas of future work, based on our interests, which are covered by the 2 points indicated above. Please keep in mind that any work is to be directly linked to a regulatory outcome, and this is where I would probably provide guidance. Thanks.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Friday, July 17, 2015 11:05 AM

To: Katz, Linda

Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Larry Walker

Subject: Re: list

Dear Linda

Thanks for quick response. Our meeting will end on 12th evening, we can stay next day and have meeting on 13th and return in the afternoon or evening back.

We have provided ppts but if you think it will be useful to give an overview, I can present the same presentation which I gave during your visit.

Nakissa, please let me know if you have some thoughts about the agenda or you would like to cover any specific topic

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Friday, July 17, 2015 at 7:24 AM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R"

<Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>

Subject: RE: list

Ikhlas,

I will be out of the office when you are here in August. However, Nakissa and Stan would love to meet with you and will probably also invite our colleagues in OARSA, who are doing related research, to attend for a broader discussion of methods and direction. Let me know your actual availability, including the amount of time you will have to spend with us so that we can make arrangements for a room. Also let me know if you will be making any formal presentations. We will work on an agenda from our end which we will share. Because I will be out of the office from August 3 through August 13, Nakissa will be your point of contact.

Linda

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Thursday, July 16, 2015 12:15 PM

To: Katz, Linda

Cc: Sadrieh, Nakissa; Milstein, Stanley R; AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO

Subject: Re: list

Dear Linda

During your visit we discussed a possibility to meet the OCAC group during our visit to CFSAN in August. We have COE meeting on August 12th. We can plan to meet on 13th if its feasible.

Let me know if we would like to meet and we will make our return travel plan accordingly.

ik

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Friday, July 10, 2015 at 10:45 AM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, "Milstein, Stanley R" <Stanley.Milstein@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, CRISTINA AVONTO <cavonto@olemiss.edu>
Subject: RE: list

Ikhlas,

Thanks so much for this information. We will be back in touch.

Have a nice weekend as well.

Linda

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
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Subject: FW: list

Ikhlas,

Attached is the list of the 26 EU fragrance allergens as well as the SCCS opinion. Some of these substances are either confirmed contact allergens or presumptive contact allergens (but not confirmed); other tables present those which are suspected to be human contact allergens or which are based on animal, in-vitro (LLNA), or QSAR/*in-silico* data. Table 13-5 presents 12 established chemical fragrance allergens with high risk of sensitization to humans. In the SCCS Opinion, the Threshold exposure (for single chemicals only, not extracts or mixtures) which could be tolerated by most consumers is estimated to be = 0.8 µg/cm².

Chemical of special concern include: tree moss, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), oak moss, isoeugenol, hydroxycitronellal, citral, cinnamal, farnesol and cinnamyl alcohol. However, we are interested in the remainder of those listed below from Table 13-5.

Cinnamal
Cinnamyl Alcohol*
Citral
Coumarin
Eugenol*
Farnesol*
Geraniol*
Hydroxycitronellal
Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC)
Isoeugenol*
Limonene (oxidised)
Linalool* (oxidised)
*including their respective esters

The 2012 SCCS Opinion also recommends that fragrance pre-haptens and pro-haptens of several terpene fragrance materials should be considered as putative "allergens" and regulated in the same way as the allergens by the EC (Among them are limonene, linalool, linalyl acetate, geraniol, geranial, α-terpinene, eugenol, isoeugenol, and cinnamyl alcohol).

Let me know if you need any further information at this time regarding allergens.

On a different note, let me know if you have a report and list with description of in vitro assays, including validation, that actually describes the methods that you have developed for arbutin.

Linda

From: Hansen, Patricia A
Sent: Wednesday, June 17, 2015 1:21 PM
To: Ikhlas Khan
Cc: Katz, Linda
Subject: RE: list

Hi, Ikhlas. Sorry not to get back to you sooner.

I think the conversations we had while on our visit were very helpful.

By copy of this note, I'll alert Linda to your request for the fragrance allergen list. We talked about it during our visit and she may have assigned it to someone already, but I'm not sure.

Is there a conference call already scheduled, perhaps with the dietary supplement group and others or are you talking about something else?

Hope all is well.

PH

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Friday, June 05, 2015 10:58 AM
To: Hansen, Patricia A
Subject: list

Hi Pat

I know this is your first week and must be busy. Could you please share the list of 26 and may be 80 if possible.

It will help to us to see what is coming but you will still have time to prioritize before we have conference call

Thanks

IK

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: Manuscript
Date: Thursday, December 08, 2016 10:23:56 PM

Thanks, appreciate it. I know its busy time and you have lot going on.

Appreciate your help

ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Thursday, December 8, 2016 at 8:51 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Subject: Manuscript

Hi Ikhlas,

I spoke with Linda and she said that she has given Stan until next Wednesday to finish his review of the manuscript. Hopefully, you will hear from Stan or Linda next week. We are sorry for the delay, but we have been very busy, because of the transition in the government, and we have had a lot of increased activity. Thank you for your patience.

Nakissa.

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: Manuscript
Date: Thursday, December 08, 2016 10:52:38 PM

Please send your edits, it will help
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Thursday, December 8, 2016 at 9:34 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Re: Manuscript

I sent the PMR, or whatever that document is called, back to Cara today, with my edits included. She will send it to you, once she has made her own edits. If you want me to send you what I sent her, I can do that. Let me know if you want that. Thanks.

From: Ikhlas Khan <ikhlan@olemiss.edu>
Date: December 8, 2016 at 10:23:55 PM EST
To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Subject: Re: Manuscript

Thanks, appreciate it. I know its busy time and you have lot going on.
Appreciate your help
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Thursday, December 8, 2016 at 8:51 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: Manuscript

Hi Ikhlas,

I spoke with Linda and she said that she has given Stan until next Wednesday to finish his review of the manuscript. Hopefully, you will hear from Stan or Linda next week. We are sorry for the delay, but we have been very busy, because of the transition in the government, and we have had a lot of increased activity. Thank you for your patience.

Nakissa.

From: [AMAR GOPAL CHITTIBOYINA](#)
To: [Sadrieh, Nakissa](#)
Cc: [Vukmanovic, Stanislav](#)
Subject: Re: Manuscript on 24 fragrance ingredients
Date: Wednesday, June 14, 2017 8:38:03 PM

Thanks a lot, will wait for your input

Sent from Amar's iPhone

On Jun 14, 2017, at 7:30 PM, Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov> wrote:

Hello Amar,

I am sorry that I let this fall through the cracks. Stan is in vacation now, so I will try to review this and when Stan comes back, he can also review the manuscript. We will send this back by the end of June if that's OK.

From: Amar Chittiboyina <amar@olemiss.edu>
Date: June 14, 2017 at 8:26:17 PM EDT
To: Vukmanovic, Stanislav <Stanislav.Vukmanovic@fda.hhs.gov>, Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Subject: RE: Manuscript on 24 fragrance ingredients

Hello Nakissa,
Did you get a chance to review the contents on stability and reactivity of 24 ingredients? Thanks, -Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Wednesday, May 31, 2017 4:48 PM
To: Sadrieh, Nakissa (Nakissa.Sadrieh@fda.hhs.gov); Stanislav.Vukmanovic@fda.hhs.gov
Cc: Ikhlas A. Khan (ikh@olemiss.edu); Cristina Avonto (cavonto@olemiss.edu)
Subject: Manuscript on 24 fragrance ingredients

Dear Nakissa and Stan,

Draft on stability and reactivity of 24 fragrance ingredients using in-chemico (DCYA) method is attached. Please review the manuscript and let us know when we can submit it to toxicology journals and your input is very valuable. At this point, we are thinking to send it to Toxicology In vitro. If you see any other journal as a better fit, let us know.

Thanks a lot,

Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: [Amar Chittiboyina](#)
To: [Sadrieh, Nakissa](#); [Vukmanovic, Stanislav](#)
Subject: RE: Manuscript on 24 fragrance ingredients
Date: Wednesday, June 14, 2017 8:26:18 PM

Hello Nakissa,

Did you get a chance to review the contents on stability and reactivity of 24 ingredients? Thanks, -
Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: Amar Chittiboyina [mailto:amar@olemiss.edu]

Sent: Wednesday, May 31, 2017 4:48 PM

To: Sadrieh, Nakissa (Nakissa.Sadrieh@fda.hhs.gov); Stanislav.Vukmanovic@fda.hhs.gov

Cc: Ikhlas A. Khan (ikhlan@olemiss.edu); Cristina Avonto (cavonto@olemiss.edu)

Subject: Manuscript on 24 fragrance ingredients

Dear Nakissa and Stan,

Draft on stability and reactivity of 24 fragrance ingredients using in-chemico (DCYA) method is attached. Please review the manuscript and let us know when we can submit it to toxicology journals and your input is very valuable. At this point, we are thinking to send it to Toxicology In vitro. If you see any other journal as a better fit, let us know.

Thanks a lot,

Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: [Amar Chittiboyina](#)
To: [Vukmanovic, Stanislav](#)
Cc: [Sadrieh, Nakissa](#); [Ikhlas A. Khan](#); [Cristina Avonto](#)
Subject: RE: Manuscript on 24 fragrance ingredients
Date: Friday, June 30, 2017 4:22:12 PM
Attachments: [dcya_fragrances draft 06302017.docx](#)
[Supp_Info_24 Compounds.pdf](#)

Hi Stan,

See below for our response to your queries (highlighted in red). To make it easy, previous track changes were accepted.

- 1) In regards to citronellol “erratic data”, reading the paper I understood that degradation data was erratic, not the DCYA data. In any case, could you please also send us the file with supplementary data? I would like to see it, and I think it will be required for clearance, as well.

Supplementary data file is attached.

- 2) I don’t understand why anisyl alcohol was declared positive after degradation. Its DCYA activity (in Fig. 5) is overall very similar to cinnamyl alcohol, hexyl cinnamal, amyl cinammyl alcohol and benzyl benzoate, all of which are declared negative. Although I am judging this based on perceived height of bars (and not actual numbers), some bars look very, very similar (e.g. day 92 anisyl alcohol and day 29 benzyl benzoate). This is why my count for positive was 14 and yours was 15. Please review and reconsider.

Due to increase in reactivity with minimal standard deviation, we have included Anisyl alcohol. You are correct, and just to avoid further confusion, we have omitted the Anisyl alcohol from the list and revised manuscript accordingly.

- 3) Thank you for your explanation of classification of DCYA reactivity (comment CA10). I still think this information needs to find place in the text for the reader, and not be directed only to me (albeit I appreciate it 😊).

Agree with you and you raised very good point. Revised the manuscript accordingly.

- 4) Finally, with regards to Nakissa’s authorship, I spoke to her about it. While we both understand and appreciate your argument about “team efforts”, she feels that different members of the team contribute to different degrees to various portions of the overall project, and that manuscript authorship should reflect contributions to that particular portion. Hence, she feels it would be unethical from her part to accept the authorship on this paper with no contributions to it. As mentioned earlier, she will be looking forward to the authorship on the projects that she has been involved with. Thanks for understanding.

We respect her decision and looking forward to work with you closely. Two other manuscripts are in draft stage and we would like to see Nakissa as an author.

Thank you and have a good, long weekend.

Amar

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]

Sent: Wednesday, June 28, 2017 5:56 PM

To: Vukmanovic, Stanislav

Subject: RE: Manuscript on 24 fragrance ingredients

Dear Stan,

Revised copy with our responses to your concerns is attached. Once you are satisfied, go ahead and submit for your office clearance. Will be waiting for your e-mail before we submit to journal.

Thanks,

Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

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From: Vukmanovic, Stanislav [<mailto:Stanislav.Vukmanovic@fda.hhs.gov>]

Sent: Monday, June 26, 2017 9:17 AM

To: Amar Chittiboyina

Subject: RE: Manuscript on 24 fragrance ingredients

Hi Amar,

I will certainly ask Nakissa to reconsider, since you put it that way. When you revise, please send it to me again. If everything is OK, only then I will be able to send it for office clearance (only final docs can be cleared, unfortunately).

Looking forward to hear from you,

Stan

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]

Sent: Monday, June 26, 2017 9:44 AM

To: Vukmanovic, Stanislav

Cc: Ikhlas A. Khan

Subject: RE: Manuscript on 24 fragrance ingredients

Stan,

Thank for your critique and valuable suggestions, we will address them meticulously. Would you like to re-review after incorporating the changes? Is it cleared from your side? Shall we go ahead and submit to journal?

It's not question of how much is each contributed, rather we want to reflect as a "team" to address the safety concerns of fragrance ingredients and Nakissa is fully involved in our research activities. Can you ask her to re-consider the decision?

Thanks,
Amar

Amar Chittiboyina, PhD

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From: Vukmanovic, Stanislav [<mailto:Stanislav.Vukmanovic@fda.hhs.gov>]
Sent: Monday, June 26, 2017 7:16 AM
To: Amar Chittiboyina; Sadrieh, Nakissa
Cc: Ikhlas A. Khan; Cristina Avonto
Subject: RE: Manuscript on 24 fragrance ingredients

Dear Amar,

Thank you very much for your draft. I found the topic very interesting and enjoyed reading it. I have made some comments and added a paragraph or so to reflect my thoughts on how these results may be interpreted. I hope that you will find the comments helpful and that added text will be acceptable to you.

Also, Nakissa does not feel she has contributed to this manuscript sufficiently to warrant authorship, but is looking forward to future papers on projects we recently discussed.

Thank you,

Stan

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Wednesday, May 31, 2017 5:48 PM
To: Sadrieh, Nakissa; Vukmanovic, Stanislav
Cc: Ikhlas A. Khan; Cristina Avonto
Subject: Manuscript on 24 fragrance ingredients

Dear Nakissa and Stan,

Draft on stability and reactivity of 24 fragrance ingredients using in-chemico (DCYA) method is attached. Please review the manuscript and let us know when we can submit it to toxicology journals and your input is very valuable. At this point, we are thinking to send it to Toxicology In vitro. If you see any other journal as a better fit, let us know.

Thanks a lot,

Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

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pharmacy.olemiss.edu/ncnpr/

From: [Amar Chittiboyina](#)
To: [Vukmanovic, Stanislav](#); [Sadrieh, Nakissa](#)
Cc: ["Ikhlas A. Khan"](#)
Subject: RE: Manuscript on Ascaridole, component of tea tree oil
Date: Friday, May 06, 2016 6:51:45 PM

Thank you Stan, for the quick response with thoughtful comments. Addressed all your questions and submitted to Chemical Research in Toxicology. Thank you once again.

-Amar

From: Vukmanovic, Stanislav [mailto:Stanislav.Vukmanovic@fda.hhs.gov]
Sent: Thursday, May 05, 2016 2:17 PM
To: Amar Chittiboyina; Sadrieh, Nakissa
Cc: Ikhlas A. Khan
Subject: RE: Manuscript on Ascaridole, component of tea tree oil

Hi Amar,

Thank you for the paper, it's good to know what you have been up to. We feel that no one at OCAC has been sufficiently involved in this work to warrant authorship. So, please, go ahead and submit it without us. Thanks for asking, though. I have made some comments in the paper (attached), more of a general science reader type, than a chemistry expert type. I hope they will still be helpful. Good luck with submission!

Best,

Stan

From: Amar Chittiboyina [mailto:amar@olemiss.edu]
Sent: Thursday, May 05, 2016 10:48 AM
To: Sadrieh, Nakissa; Vukmanovic, Stanislav
Cc: Ikhlas A. Khan
Subject: Manuscript on Ascaridole, component of tea tree oil

Dear Nakissa and Stan,

As a part of our ongoing studies on tea tree oil (TTO), we identified possible degradation of ascaridole to reactive species via our *in chemico* method, HTS-DCYA. The attached manuscript is on 'what happens after activation of ascaridole' and implications to TTO. As you know, there are several clinical studies were reported on skin-sensitization of ascaridole and ascaridole containing essential oils and we believe that the identification of such intermediates are very important in assessing the sensitization capacity of TTO.

We are planning to submit it to Chemical Research in Toxicology as a rapid reports. Can you comment on the content and overall findings included in the manuscript? Also, would you suggest us the authors from OCC to include in the manuscript?

Thank you

Amar G. Chittiboyina, PhD
Senior Research Scientist
3040 National Center for Natural Product Research
University, MS 38677
662.915.1572 (off)
662.915.7989 (fax)

From: [Ikhlas Khan](#)
To: [Katz, Linda](#)
Cc: [Sadrieh, Nakissa](#); [AMAR GOPAL CHITTIBOYINA](#); [Milstein, Stanley R](#)
Subject: Re: Manuscripts
Date: Thursday, December 01, 2016 1:03:58 PM

Thanks, appreciate it
ik

From: "Katz, Linda"
Date: Thursday, December 1, 2016 at 12:01 PM
To: ikhlas
Cc: "Sadrieh, Nakissa" , [AMAR GOPAL CHITTIBOYINA](#) , "Milstein, Stanley R"
Subject: RE: Manuscripts

Ikhlas,
It is still with Stan Milstein. Stan will hopefully complete the review by the end of this week or early next week.
Linda

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Thursday, December 01, 2016 12:33 PM
To: Katz, Linda
Cc: Sadrieh, Nakissa; [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: Manuscripts

Dear Linda

Hope you all had a good break for thanksgiving. This is just a request for the manuscript , I know you have more important things to do but I will appreciate your help
IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Friday, November 18, 2016 at 12:18 PM
To: ikhlas <ikhlan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, [AMAR GOPAL CHITTIBOYINA](#) <amar@olemiss.edu>
Subject: Re: Manuscripts

Iklhlas

Stan Milstein provided comments from both of us to Diego about 2 weeks ago. Diego has not returned a corrected manuscript for clearance. We're waiting.

Linda

Sent from my BlackBerry 10 smartphone.

From: Ikhlas Khan
Sent: Friday, November 18, 2016 12:42 PM
To: Katz, Linda
Cc: Sadrieh, Nakissa; [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: Manuscripts

Dear Linda

I know you are busy and do not want to disturb you but I would like to remind you about the manuscript. I had a chance to discuss about it with Nakissa during our visit. I will appreciate any

feedback you can provide.
Appreciate your help as always
IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Thursday, October 13, 2016 at 12:59 PM
To: ikhlas <ikhlan@olemiss.edu>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: RE: Manuscripts
Thanks

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Thursday, October 13, 2016 1:58 PM
To: Katz, Linda; AMAR GOPAL CHITTIBOYINA
Subject: Re: Manuscripts
Hi Linda

I understand, I don't think there is a deadline. You can clear it when you get a chance in near future
Thanks for all your support
ik

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Thursday, October 13, 2016 at 12:45 PM
To: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, ikhlas <ikhlan@olemiss.edu>
Subject: RE: Manuscripts

Thanks. We are currently swamped with things that need to get out. Is there a deadline for when you need to have the manuscript cleared? We are still working on it but there are a number of things that need to be corrected before it is cleared.

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Thursday, October 13, 2016 12:30 PM
To: 'Ikhlas Khan'; Katz, Linda
Subject: RE: Manuscripts

Dr. Katz:
Yes, the only pending and final paper (with Diego) is about chamomile, i.e., entry #7. The work on tea tree oil was published in couple of months back (June10th 2016).
Please let us know if you need any additional information.
Thanks
Amar

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Thursday, October 13, 2016 11:18 AM
To: Katz, Linda
Cc: AMAR GOPAL CHITTIBOYINA
Subject: Re: Manuscripts
#7 is pending. Let me ask Amar to clarify and confirm what is pending or what is left to be published.
IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Thursday, October 13, 2016 at 11:14 AM
To: ikhlas <ikhlan@olemiss.edu>
Subject: RE: Manuscripts
Which is the paper that is pending? In the past 3 months I have received 2 manuscripts.
Linda

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Thursday, October 13, 2016 12:11 PM

To: Katz, Linda

Subject: Re: Manuscripts

Dear Linda

A total of 6 papers were published as result of collaborative efforts from OCAC and Dr. Rua and one paper to be communicated. Of these published papers, two were related to arbutin in cosmetics¹ and enzymatic stability of arbutin². Two papers were published on 'development of alternative in-chemico methods' using multiwell plate³ reader and NMR spectroscopy⁴. These two methods were applied on tea tree oil⁵ as well as chamomile^{6,7} for identification of potential skin sensitizers.

[1] Wang, Y.-H.; Avonto, C.; Avula, B.; Wang, M.; **Rua, D.**; Khan, I. A., Quantitative determination of α -arbutin, β -arbutin, kojic acid, nicotinamide, hydroquinone, resorcinol, 4-methoxyphenol, 4-ethoxyphenol, and ascorbic acid from skin whitening products by HPLC-UV. J. AOAC Int. **2015**, 98 (1), 5-12.

[2] Avonto, C.; Wang, Y. H.; Avula, B.; Wang, M.; **Rua, D.**; Khan, I. A., Comparative studies on the chemical and enzymatic stability of α - and β -arbutin. Int. J. Cosmet. Sci. 2016, 38 (2), 187-193.

[3] Avonto, C.; Chittiboyina, A. G.; **Rua, D.**; Khan, I. A., A fluorescence high throughput screening method for the detection of reactive electrophiles as potential skin sensitizers. Toxicol. Appl. Pharmacol. **2015**, 289 (2), 177-184.

[4] Chittiboyina, A. G.; Avonto, C.; **Rua, D.**; Khan, I. A., Alternative Testing Methods for Skin Sensitization: NMR Spectroscopy for Probing the Reactivity and Classification of Potential Skin Sensitizers. Chem. Res. Toxicol. **2015**, 28 (9), 1704-1714.

[5] Avonto, C.; Chittiboyina, A. G.; Wang, M.; Vasquez, Y.; **Rua, D.**; Khan, I. A., In Chemico Evaluation of Tea Tree Essential Oils as Skin Sensitizers: Impact of the Chemical Composition on Aging and Generation of Reactive Species. Chem. Res. Toxicol. **2016**, 29 (7), 1108-1117.

[6] Avula, B.; Wang, Y.-H.; Wang, M.; Avonto, C.; Zhao, J.; Smillie, T. J.; **Rua, D.**; Khan, I. A., Quantitative determination of phenolic compounds by UHPLC-UV-MS and use of partial least-square discriminant analysis to differentiate chemo-types of Chamomile/Chrysanthemum flower heads. J. Pharm. Biomed. Anal. **2014**, 88, 278-288.

[7] Avonto, C.; **Rua, D.**; Lasonkar, P.; Chittiboyina, A. G.; Khan, I. A. Identification of a compound isolated from German chamomile (*Matricaria chamomilla*) with dermal sensitization potential. Toxicol. Appl. Pharmacol. (To be communicated)

Only one paper is pending the rest is already published.

Please let me know if you need further information or want to discuss

ik

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Thursday, October 13, 2016 at 6:35 AM

To: ikhlas <ikhlan@olemiss.edu>

Subject: Re: Manuscripts

Thanks

Sent from my BlackBerry 10 smartphone.

From: Ikhlas Khan

Sent: Wednesday, October 12, 2016 10:27 PM

To: Katz, Linda

Cc: AMAR GOPAL CHITTIBOYINA

Subject: Re: Manuscripts

I will send you the list of all what we have published with him and if any pending articles.
ik

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Wednesday, October 12, 2016 at 9:25 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Subject: Re: Manuscripts

Thanks. How many manuscripts still need clearance? I am a bit concerned about what he has forwarded since he has not kept us informed since he left OCAC.

Sent from my BlackBerry 10 smartphone.

From: Ikhlas Khan

Sent: Wednesday, October 12, 2016 10:17 PM

To: Katz, Linda

Cc: Sadrieh, Nakissa; AMAR GOPAL CHITTIBOYINA

Subject: Re: Manuscripts

Dear Linda

Sorry for the late response. I got back this Sunday. All the manuscript you are seeing, these were pending manuscripts. He was involved with Arbutin, chamomile and tea tree oil work. He is not involved with any new project since he moved to other department. Yes, he did contribute to the work that he got authorship.

Since he moved from OCAC, he is not involved in any research or contribute anymore.

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Friday, October 7, 2016 at 3:08 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Subject: Manuscripts

Ikhlas,

Hope all is well with you. I have been getting quite a few manuscripts for clearance from Diego Rua over the past few weeks. Please let me know if Diego continues to be involved in any projects that he had started while he was in OCAC. Also, let me know if his contributions were significant enough to warrant authorship. Look forward to hearing from you.

Linda

Linda M. Katz, M.D., M.P.H.

Director, Office of Cosmetics and Colors

Acting Chief Medical Officer Center for Food Safety and Applied Nutrition

Food and Drug Administration

5001 Campus Drive, HFS-100

College Park, Maryland 20740

240-402-1130 (phone)

301-436-2976 (fax)

linda.katz@fda.hhs.gov

From: [Ikhlas Khan](#)
To: [Katz, Linda](#)
Cc: [Sadrieh, Nakissa](#); [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: Manuscripts
Date: Thursday, December 01, 2016 12:33:32 PM

Dear Linda

Hope you all had a good break for thanksgiving. This is just a request for the manuscript , I know you have more important things to do but I will appreciate your help

IK

From: "Katz, Linda"

Date: Friday, November 18, 2016 at 12:18 PM

To: ikhlas

Cc: "Sadrieh, Nakissa" , AMAR GOPAL CHITTIBOYINA

Subject: Re: Manuscripts

[Iklhlas](#)

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[Sent from my BlackBerry 10 smartphone.](#)

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Sent: Friday, November 18, 2016 12:42 PM

To: Katz, Linda

Cc: Sadrieh, Nakissa; AMAR GOPAL CHITTIBOYINA

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Appreciate your help as always

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From: "Katz, Linda"

Date: Thursday, October 13, 2016 at 12:59 PM

To: ikhlas , AMAR GOPAL CHITTIBOYINA

Subject: RE: Manuscripts

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Sent: Thursday, October 13, 2016 1:58 PM

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Date: Thursday, October 13, 2016 at 12:45 PM

To: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, ikhlas <ikhlan@olemiss.edu>

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From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Friday, October 7, 2016 at 3:08 PM

To: Ikhlas Khan <ikh@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

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linda.katz@fda.hhs.gov

From: [Ikhlas Khan](#)
To: [Katz, Linda](#)
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Date: Friday, November 18, 2016 2:35:00 PM

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From: [Ikhlas Khan](#)
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Cc: [Sadrieh, Nakissa](#)
Subject: Re: Manuscripts
Date: Thursday, December 01, 2016 2:26:40 PM

Appreciate your time and help
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From: "Milstein, Stanley R"
Date: Thursday, December 1, 2016 at 12:32 PM
To: ikhlas
Cc: "Sadrieh, Nakissa"
Subject: FW: Manuscripts
Concur with Dr. Katz's comments. Diego gave a very nice presentation of his work ! (see my response, below)
Thanks.

Stan Milstein

From: Milstein, Stanley R
Sent: Thursday, December 01, 2016 1:30 PM
To: Katz, Linda
Subject: RE: Manuscripts
Linda ---

Review will be completed and hopefully clearance given, today ! Thanks for your patience. I regret the minor delay.
Stan

From: Katz, Linda
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To: Milstein, Stanley R
Subject: FW: Manuscripts
Importance: High

Please complete the review and sign-off. If Diego answered all of your and my questions, I don't need to review it. Just let me know.
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Linda

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Thursday, December 01, 2016 12:33 PM
To: Katz, Linda
Cc: Sadrieh, Nakissa; AMAR GOPAL CHITTIBOYINA
Subject: Re: Manuscripts
Dear Linda

Hope you all had a good break for thanksgiving. This is just a request for the manuscript , I know you have more important things to do but I will appreciate your help

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Friday, November 18, 2016 at 12:18 PM
To: ikhlas <ikhlan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: Re: Manuscripts

Iklhlas

Stan Milstein provided comments from both of us to Diego about 2 weeks ago. Diego has not returned a corrected manuscript for clearance. We're waiting.

Linda

Sent from my BlackBerry 10 smartphone.

From: Ikhlas Khan
Sent: Friday, November 18, 2016 12:42 PM
To: Katz, Linda
Cc: Sadrieh, Nakissa; AMAR GOPAL CHITTIBOYINA
Subject: Re: Manuscripts

Dear Linda

I know you are busy and do not want to disturb you but I would like to remind you about the manuscript. I had a chance to discuss about it with Nakissa during our visit. I will appreciate any feedback you can provide.

Appreciate your help as always

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Thursday, October 13, 2016 at 12:59 PM
To: ikhlas <ikhlan@olemiss.edu>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: RE: Manuscripts

Thanks

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Thursday, October 13, 2016 1:58 PM
To: Katz, Linda; AMAR GOPAL CHITTIBOYINA
Subject: Re: Manuscripts

Hi Linda

I understand, I don't think there is a deadline. You can clear it when you get a chance in near future

Thanks for all your support
ik

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Thursday, October 13, 2016 at 12:45 PM

To: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, ikhlas <ikhlan@olemiss.edu>

Subject: RE: Manuscripts

Thanks. We are currently swamped with things that need to get out. Is there a deadline for when you need to have the manuscript cleared? We are still working on it but there are a number of things that need to be corrected before it is cleared.

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]

Sent: Thursday, October 13, 2016 12:30 PM

To: 'Ikhlas Khan'; Katz, Linda

Subject: RE: Manuscripts

Dr. Katz:

Yes, the only pending and final paper (with Diego) is about chamomile, i.e., entry #7. The work on tea tree oil was published in couple of months back (June 10th 2016).

Please let us know if you need any additional information.

Thanks

Amar

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Thursday, October 13, 2016 11:18 AM

To: Katz, Linda

Cc: AMAR GOPAL CHITTIBOYINA

Subject: Re: Manuscripts

#7 is pending. Let me ask Amar to clarify and confirm what is pending or what is left to be published.

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Thursday, October 13, 2016 at 11:14 AM

To: ikhlas <ikhlan@olemiss.edu>

Subject: RE: Manuscripts

Which is the paper that is pending? In the past 3 months I have received 2 manuscripts.

Linda

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Thursday, October 13, 2016 12:11 PM

To: Katz, Linda

Subject: Re: Manuscripts

Dear Linda

A total of 6 papers were published as result of collaborative efforts from OCAC and Dr. Rua and one paper to be communicated. Of these published papers, two were related to arbutin in cosmetics¹ and enzymatic stability of arbutin². Two papers were published on 'development of alternative in-chemico methods' using multiwell plate³ reader and NMR spectroscopy⁴. These two methods were applied on tea tree oil⁵ as well as chamomile^{6,7} for identification of potential skin sensitizers.

[1] Wang, Y.-H.; Avonto, C.; Avula, B.; Wang, M.; **Rua, D.**; Khan, I. A., Quantitative determination of a-arbutin, β -arbutin, kojic acid, nicotinamide, hydroquinone, resorcinol, 4-methoxyphenol, 4-ethoxyphenol, and ascorbic acid from skin whitening products by HPLC-UV. J. AOAC Int. **2015**, 98 (1), 5-12.

[2] Avonto, C.; Wang, Y. H.; Avula, B.; Wang, M.; **Rua, D.**; Khan, I. A., Comparative studies on the chemical and enzymatic stability of alpha- and beta-arbutin. *Int. J. Cosmet. Sci.* 2016, 38 (2), 187-193.

[3] Avonto, C.; Chittiboyina, A. G.; **Rua, D.**; Khan, I. A., A fluorescence high throughput screening method for the detection of reactive electrophiles as potential skin sensitizers. *Toxicol. Appl. Pharmacol.* **2015**, 289 (2), 177-184.

[4] Chittiboyina, A. G.; Avonto, C.; **Rua, D.**; Khan, I. A., Alternative Testing Methods for Skin Sensitization: NMR Spectroscopy for Probing the Reactivity and Classification of Potential Skin Sensitizers. *Chem. Res. Toxicol.* **2015**, 28 (9), 1704-1714.

[5] Avonto, C.; Chittiboyina, A. G.; Wang, M.; Vasquez, Y.; **Rua, D.**; Khan, I. A., In Chemico Evaluation of Tea Tree Essential Oils as Skin Sensitizers: Impact of the Chemical Composition on Aging and Generation of Reactive Species. *Chem. Res. Toxicol.* **2016**, 29 (7), 1108-1117.

[6] Avula, B.; Wang, Y.-H.; Wang, M.; Avonto, C.; Zhao, J.; Smillie, T. J.; **Rua, D.**; Khan, I. A., Quantitative determination of phenolic compounds by UHPLC-UV-MS and use of partial least-square discriminant analysis to differentiate chemo-types of Chamomile/Chrysanthemum flower heads. *J. Pharm. Biomed. Anal.* **2014**, 88, 278-288.

[7] Avonto, C.; **Rua, D.**; Lasonkar, P.; Chittiboyina, A. G.; Khan, I. A. Identification of a compound isolated from German chamomile (*Matricaria chamomilla*) with dermal sensitization potential. *Toxicol. Appl. Pharmacol.* (To be communicated)

Only one paper is pending the rest is already published.

Please let me know if you need further information or want to discuss

ik

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Thursday, October 13, 2016 at 6:35 AM

To: ikhlas <ikhlan@olemiss.edu>

Subject: Re: Manuscripts

Thanks

Sent from my BlackBerry 10 smartphone.

From: Ikhlas Khan

Sent: Wednesday, October 12, 2016 10:27 PM

To: Katz, Linda

Cc: AMAR GOPAL CHITTIBOYINA

Subject: Re: Manuscripts

I will send you the list of all what we have published with him and if any pending articles.

ik

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Wednesday, October 12, 2016 at 9:25 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Subject: Re: Manuscripts

Thanks. How many manuscripts still need clearance? I am a bit concerned about what he has forwarded since he has not kept us informed since he left OCAC.

Sent from my BlackBerry 10 smartphone.

From: Ikhlas Khan

Sent: Wednesday, October 12, 2016 10:17 PM

To: Katz, Linda

Cc: Sadrieh, Nakissa; AMAR GOPAL CHITTIBOYINA

Subject: Re: Manuscripts

Dear Linda

Sorry for the late response. I got back this Sunday. All the manuscript you are seeing, these were pending manuscripts. He was involved with Arbutin, chamomile and tea tree oil work. He is not involved with any new project since he moved to other department. Yes, he did contribute to the work that he got authorship.

Since he moved from OCAC, he is not involved in any research or contribute anymore.

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>

Date: Friday, October 7, 2016 at 3:08 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Subject: Manuscripts

Ikhlas,

Hope all is well with you. I have been getting quite a few manuscripts for clearance from Diego Rua over the past few weeks. Please let me know if Diego continues to be involved in any projects that he had started while he was in OCAC. Also, let me know if his contributions were significant enough to warrant authorship. Look forward to hearing from you.

Linda

Linda M. Katz, M.D., M.P.H.

Director, Office of Cosmetics and Colors

Acting Chief Medical Officer Center for Food Safety and Applied Nutrition

Food and Drug Administration

5001 Campus Drive, HFS-100

College Park, Maryland 20740

240-402-1130 (phone)

301-436-2976 (fax)

linda.katz@fda.hhs.gov

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#); [AMAR GOPAL CHITTIBOYINA](#)
Cc: [Moghaddam, Sarvin](#); [Vukmanovic, Stanislav](#)
Subject: Re: Meeting today
Date: Thursday, March 09, 2017 2:36:33 PM

Good. Yes, please let us know when you are ready.

ik

From: "Sadrieh, Nakissa"
Date: Thursday, March 9, 2017 at 11:13 AM
To: ikhlas , AMAR GOPAL CHITTIBOYINA
Cc: "Moghaddam, Sarvin" , "Vukmanovic, Stanislav"
Subject: FW: Meeting today

Hi Ikhlas,

I just wanted to respond to the email that you just me, and to let you know that we have receive Amar's email, with the attachments. we will get back to you shortly, so that we can get started with the studies.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Friday, March 03, 2017 3:45 PM
To: Sadrieh, Nakissa; 'Ikhlas Khan'
Cc: 'CRISTINA AVONTO'; Moghaddam, Sarvin; Vukmanovic, Stanislav; Shabana Khan
Subject: RE: Meeting today

Hello Nakissa,

Thank you for the call and it is helpful for us to identify the priorities and goals. As you suggested, we will schedule the teleconference calls for every two months to abreast our research efforts.

In addition to the DPRA report on 24 ingredients, the protocols we are implementing at NCNPR is also attached. Let us know if you need any additional information.

Thanks,

Amar

From: Sadrieh, Nakissa [<mailto:Nakissa.Sadrieh@fda.hhs.gov>]
Sent: Friday, February 17, 2017 10:27 AM
To: 'Ikhlas Khan'
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Moghaddam, Sarvin; Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Do you have a telephone number that we can call? We can try to find a conference room, and then call you. Sarvin and Stan will be joining the discussion. if you want to call us, our conference room

telephone number is (b) (6) . We will likely use that line. thank you.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Friday, February 17, 2017 10:05 AM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: Meeting today
Sure, lets do at 4:00 to be safe.
Which number should we call
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Friday, February 17, 2017 at 7:07 AM
To: ikhlas <ikhlan@olemiss.edu>
Subject: Meeting today

Ikhlas,

We have had a meeting scheduled at 2 pm on the other campus, and I won't be back till 3:30. The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: meeting today
Date: Tuesday, November 22, 2016 11:24:17 AM

Hi Nakissa

You were talking about Aloe and here it is
ik

From: "Sadrieh, Nakissa"

Date: Thursday, October 27, 2016 at 10:38 AM

To: ikhlas

Subject: meeting today

Ikhlas,

Can I stop by the cafeteria at 12:45? Please start eating, since I need to finish something before I come there. I will eat something in my office before I come there. if I am still hungry, I will get something else to eat to keep you company. thanks.

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#); [CRISTINA AVONTO](#); [Moghaddam, Sarvin](#); [Vukmanovic, Stanislav](#); [Jennifer S. Taylor](#)
Subject: Re: Meeting today
Date: Friday, February 17, 2017 11:32:57 AM

Jennifer will try to get conference call# shortly

From: "Sadrieh, Nakissa"

Date: Friday, February 17, 2017 at 10:27 AM

To: ikhlas

Cc: AMAR GOPAL CHITTIBOYINA , CRISTINA AVONTO , "Moghaddam, Sarvin" , "Vukmanovic, Stanislav" , "Sadrieh, Nakissa"

Subject: RE: Meeting today

Do you have a telephone number that we can call? We can try to find a conference room, and then call you. Sarvin and Stan will be joining the discussion. if you want to call us, our conference room telephone number is (b) (6) . We will likely use that line. thank you.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [mailto:ikhan@olemiss.edu]
Sent: Friday, February 17, 2017 10:05 AM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: Meeting today
Sure, lets do at 4:00 to be safe.
Which number should we call
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Friday, February 17, 2017 at 7:07 AM

To: ikhlas <ikhan@olemiss.edu>

Subject: Meeting today

Ikhlas,

We have had a meeting scheduled at 2 pm on the other campus, and I won't be back till 3:30. The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: meeting today
Date: Thursday, October 27, 2016 12:34:50 PM

Thats fine

Sent from my iPhone

On Oct 27, 2016, at 11:38 AM, Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov> wrote:

Ikhlas,

Can I stop by the cafeteria at 12:45? Please start eating, since I need to finish something before I come there. I will eat something in my office before I come there. if I am still hungry, I will get something else to eat to keep you company. thanks.

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: [Amar Chittiboyina](#)
To: [Moghaddam, Sarvin](#); ["Ikhlas Khan"](#); ["CRISTINA AVONTO"](#); ["Shabana Khan"](#)
Cc: [Vukmanovic, Stanislav](#); [Sadrieh, Nakissa](#)
Subject: RE: Meeting today
Date: Friday, March 31, 2017 5:54:45 PM

Hello Sarvin,

Thank you for the update. We are in process of finalizing the data (HTS-DCYA, hCLAT) on 24 compounds, shall send it to you shortly. Meanwhile we will explore the possible sources and acquire the suggested compounds. Yes, we did not forget about Stan's proposal on Reactive chemicals, induction of ROS and assessment with KeratinoSens. Currently we are validating the KeratinoSens assay in our labs by testing the known compounds. Once the validation is complete, will explore for new ingredients as well as new ideas.

Thanks

Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [<mailto:Sarvin.Moghaddam@fda.hhs.gov>]

Sent: Friday, March 31, 2017 12:33 PM

To: Amar Chittiboyina; 'Ikhlas Khan'; 'CRISTINA AVONTO'; Shabana Khan

Cc: Vukmanovic, Stanislav; Sadrieh, Nakissa

Subject: RE: Meeting today

Hello Amar,

Thank you for the update on the project status. We have some questions and requests for the further directions of our joint projects:

- 1) Following our last meeting, our understanding was that you have hCLAT and HTS-DCYA results as well as Cys-DPRA and Lys-DPRA results for the 24 fragrance allergens. However, in the "Progress Report_Set1_24 Ingredients.doc" report provided on March 9th, only Cys-DPRA and Lys-DPRA results for the 24 fragrance allergens are provided. We would like to see if you can update the report with hCLAT and HTS-DCYA results and also KeratinoSens results at later time.
- 2) Following some discussion in our group, we would like to expand the project with some new ingredients. Attached, please find the list of ingredients including the initial 24 fragrance allergens for which available results have already been populated.
- 3) Finally, a while ago when we were talking about introducing KeratinoSens assay we discussed a project on delineating the covalent bond-mediated effects from those mediated by reactive oxygen radicals. To refresh our memories, project description is attached. Since KeratinoSens appears to be finally in place, we would like to ask you to make a plan (and send it to us) on carrying this project forward.

Thank you

Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]

Sent: Friday, March 03, 2017 3:45 PM

To: Sadrieh, Nakissa; 'Ikhlas Khan'

Cc: 'CRISTINA AVONTO'; Moghaddam, Sarvin; Vukmanovic, Stanislav; Shabana Khan

Subject: RE: Meeting today

Hello Nakissa,

Thank you for the call and it is helpful for us to identify the priorities and goals. As you suggested, we will schedule the teleconference calls for every two months to abreast our research efforts.

In addition to the DPRA report on 24 ingredients, the protocols we are implementing at NCNPR is also attached. Let us know if you need any additional information.

Thanks,

Amar

From: Sadrieh, Nakissa [<mailto:Nakissa.Sadrieh@fda.hhs.gov>]

Sent: Friday, February 17, 2017 10:27 AM

To: 'Ikhlas Khan'

Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Moghaddam, Sarvin; Vukmanovic, Stanislav; Sadrieh, Nakissa

Subject: RE: Meeting today

Do you have a telephone number that we can call? We can try to find a conference room, and then call you. Sarvin and Stan will be joining the discussion. if you want to call us, our conference room telephone number is (b) (6). We will likely use that line. thank you.

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Friday, February 17, 2017 10:05 AM

To: Sadrieh, Nakissa

Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO

Subject: Re: Meeting today

Sure, lets do at 4:00 to be safe.

Which number should we call

ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Friday, February 17, 2017 at 7:07 AM

To: ikhlas <ikhlan@olemiss.edu>

Subject: Meeting today

Ikhlas,

We have had a meeting scheduled at 2 pm on the other campus, and I won't be back till 3:30. The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#); [CRISTINA AVONTO](#)
Subject: Re: Meeting today
Date: Friday, February 17, 2017 10:06:09 AM

Sure, lets do at 4:00 to be safe.
Which number should we call
ik

From: "Sadrieh, Nakissa"
Date: Friday, February 17, 2017 at 7:07 AM
To: ikhlas
Subject: Meeting today

Ikhlas,
We have had a meeting scheduled at 2 pm on the other campus, and I won't be back till 3:30. The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

From: [Amar Chittiboyina](#)
To: [Sadrieh, Nakissa](#); ["Ikhlas Khan"](#)
Cc: ["CRISTINA AVONTO"](#); [Moghaddam, Sarvin](#); [Vukmanovic, Stanislav](#); [Shabana Khan](#)
Subject: RE: Meeting today
Date: Friday, March 03, 2017 3:46:14 PM
Attachments: [Set2_24 Compounds.xlsx](#)
[Common plant allergens_02202017.xlsx](#)
[Protocol_DPRA.DOCX](#)
[Protocol_hCLAT.DOCX](#)
[Protocol_HTS-DCYA.DOCX](#)
[Protocol_NMR-DCYA.docx](#)
[Progress Report_Set1_24 Ingredients.docx](#)

Hello Nakissa,

Thank you for the call and it is helpful for us to identify the priorities and goals. As you suggested, we will schedule the teleconference calls for every two months to abreast our research efforts.

In addition to the DPRA report on 24 ingredients, the protocols we are implementing at NCNPR is also attached. Let us know if you need any additional information.

Thanks,
Amar

From: Sadrieh, Nakissa [<mailto:Nakissa.Sadrieh@fda.hhs.gov>]
Sent: Friday, February 17, 2017 10:27 AM
To: 'Ikhlas Khan'
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Moghaddam, Sarvin; Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

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Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
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Food and Drug Administration (FDA)
4300 River Road
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From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Friday, February 17, 2017 10:05 AM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO

Subject: Re: Meeting today

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Date: Friday, February 17, 2017 at 7:07 AM

To: ikhlas <ikhlan@olemiss.edu>

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The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

From: [Amar Chittiboyina](#)
To: [Moghaddam, Sarvin](#); ["Ikhlas Khan"](#); ["CRISTINA AVONTO"](#); ["Shabana Khan"](#)
Cc: [Vukmanovic, Stanislav](#); [Sadrieh, Nakissa](#)
Subject: RE: Meeting today
Date: Thursday, April 13, 2017 6:08:37 PM
Attachments: [hCLAT final results_24compounds_April2017.xlsx](#)
[DCYA report of 24 fragrances 04112017.docx](#)

Hello Sarvin,

As per your request, the data on hCLAT and HTS-DCYA is attached. The validation of KeratinoSens is completed in our labs and we are in process of testing these 24 ingredients.

It's the time again for the conference call with your team and suggest us your convenient time.

Thank you and have a good weekend

Amar

Amar Chittiboyina, PhD

Assistant Director, NCNPR

311H TCRC West, School of Pharmacy, University, MS 38655

[Tel:+1-662-915-1572](tel:+1-662-915-1572) | [Fax:+1-662-915-7989](tel:+1-662-915-7989)

pharmacy.olemiss.edu/ncnpr/

From: Moghaddam, Sarvin [mailto:Sarvin.Moghaddam@fda.hhs.gov]
Sent: Friday, March 31, 2017 12:33 PM
To: Amar Chittiboyina; 'Ikhlas Khan'; 'CRISTINA AVONTO'; Shabana Khan
Cc: Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Hello Amar,

Thank you for the update on the project status. We have some questions and requests for the further directions of our joint projects:

- 1) Following our last meeting, our understanding was that you have hCLAT and HTS-DCYA results as well as Cys-DPRA and Lys-DPRA results for the 24 fragrance allergens. However, in the "Progress Report_Set1_24 Ingredients.doc" report provided on March 9th, only Cys-DPRA and Lys-DPRA results for the 24 fragrance allergens are provided. We would like to see if you can update the report with hCLAT and HTS-DCYA results and also KeratinoSens results at later time.
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Thank you
Sarvin

From: Amar Chittiboyina [<mailto:amar@olemiss.edu>]
Sent: Friday, March 03, 2017 3:45 PM
To: Sadrieh, Nakissa; 'Ikhlas Khan'
Cc: 'CRISTINA AVONTO'; Moghaddam, Sarvin; Vukmanovic, Stanislav; Shabana Khan
Subject: RE: Meeting today

Hello Nakissa,

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Thanks,
Amar

From: Sadrieh, Nakissa [<mailto:Nakissa.Sadrieh@fda.hhs.gov>]
Sent: Friday, February 17, 2017 10:27 AM
To: 'Ikhlas Khan'
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO; Moghaddam, Sarvin; Vukmanovic, Stanislav; Sadrieh, Nakissa
Subject: RE: Meeting today

Do you have a telephone number that we can call? We can try to find a conference room, and then call you. Sarvin and Stan will be joining the discussion. if you want to call us, our conference room telephone number is (b) (6) We will likely use that line. thank you.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Friday, February 17, 2017 10:05 AM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA; CRISTINA AVONTO
Subject: Re: Meeting today

Sure, lets do at 4:00 to be safe.
Which number should we call

ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Friday, February 17, 2017 at 7:07 AM

To: ikhlas <ikhlan@olemiss.edu>

Subject: Meeting today

Ikhlas,

We have had a meeting scheduled at 2 pm on the other campus, and I won't be back till 3:30. The meeting is in the commissioner's office, and I have to attend in person. Can we postpone our telecom to 3:30 or 3:45? Thanks.

From: [Ikhlas Khan](#)
To: [Welch, Cara](#); [AMAR GOPAL CHITTIBOYINA](#); [Sadrieh, Nakissa](#)
Subject: Re: NCNPR cooperative agreement quarterly agreement
Date: Thursday, October 13, 2016 1:55:37 PM

We are ok with it
ik

From: Cara Welch
Date: Thursday, October 13, 2016 at 12:48 PM
To: AMAR GOPAL CHITTIBOYINA , "Sadrieh, Nakissa" , ikhlas
Subject: Re: NCNPR cooperative agreement quarterly agreement

All,

I have a meeting that is scheduled until 2:30 - would you be opposed if we started our meeting at 2:30? We may get done by 3:00 anyway, if anyone has a hard stop.

Thanks

Cara

From: Welch, Cara
When: October 13, 2016 at 2:00:00 PM EDT
Required: Ikhlas Kahn , Sadrieh, Nakissa , AMAR GOPAL CHITTIBOYINA
Subject: NCNPR cooperative agreement quarterly agreement
Location: Conf call: (b) (6) Conf ID: (b) (6)

Call-in info added

Conf call: (b) (6) Conf ID: (b) (6)

From: [Amar Chittiboyina](#)
To: ["Ikhlas Khan"; Sadrieh, Nakissa](#)
Subject: RE: NCNPR-CFSAN monthly meeting
Date: Tuesday, January 24, 2017 6:22:17 PM
Attachments: [Collaborative research efforts with CFSAN.DOCX](#)
[Cosmetic Work NCNPR 012017.pdf](#)

The materials to familiarize your team are attached and let us know if you need any additional information. Thanks, -Amar

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Tuesday, January 24, 2017 4:52 PM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA
Subject: Re: NCNPR-CFSAN monthly meeting

Sounds good, IS next Friday Feb 3rd morning will work for?
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Tuesday, January 24, 2017 at 4:06 PM
To: ikhlas <ikhlan@olemiss.edu>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: RE: NCNPR-CFSAN monthly meeting

Hello,

I think that next week would be good for us to talk. can you please send me a status of the various projects and assays that you have available for cosmetics research? I have new staff that I wish to assign to working with you, and I want to familiarize them with the previous work, as well as the ongoing work and what we have discussed as planned future projects. So next week would be a good time to talk. thank you.

Regards,
Nakissa Sadrieh, Ph.D.
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Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Tuesday, January 24, 2017 4:01 PM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA
Subject: Re: NCNPR-CFSAN monthly meeting

Hi Nakissa

I know you are busy but I think we should talk about the future work whenever you have time
IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Tuesday, January 10, 2017 at 10:48 AM
To: ikhlas <ikhlan@olemiss.edu>, Cara Welch <Cara.Welch@fda.hhs.gov>, "Swift, Sibyl" <Sibyl.Swift@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Eric Olson <Eric.Olson@fda.hhs.gov>
Cc: JT <jnnfrtyl@olemiss.edu>
Subject: RE: NCNPR-CFSAN monthly meeting

I think that we can postpone the call to another time, since we are preparing for the apocalypse here (January 20th). but I look forward to a discussion after that. we have ideas for jump starting our research for 2017.

Regards,
Nakissa Sadrieh, Ph.D.
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Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Tuesday, January 10, 2017 11:06 AM
To: Welch, Cara; Swift, Sibyl; Sadrieh, Nakissa; AMAR GOPAL CHITTIBOYINA; Olson, Eric
Cc: Jennifer S. Taylor
Subject: Re: NCNPR-CFSAN monthly meeting

Nakissa, let us know if you want to me on or we can call some other time
ik

From: Cara Welch <Cara.Welch@fda.hhs.gov>

Date: Tuesday, January 10, 2017 at 7:39 AM

To: "Swift, Sibyl" <Sibyl.Swift@fda.hhs.gov>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Eric Olson <Eric.Olson@fda.hhs.gov>, ikhlas <ikhlan@olemiss.edu>

Cc: JT <jnnfrtyl@olemiss.edu>

Subject: RE: NCNPR-CFSAN monthly meeting

ODSP needs to cancel this month's meeting, Thursday, Jan 12 – OCAC or NCNPR, do you want to keep the meeting amongst yourselves?

Cara

-----Original Appointment-----

From: Welch, Cara

Sent: Thursday, October 13, 2016 3:27 PM

To: Welch, Cara; Swift, Sibyl; Sadrieh, Nakissa; Amar Chittiboyina (amar@olemiss.edu); Olson, Eric; ikhlan@olemiss.edu

Cc: Jennifer S. Taylor

Subject: NCNPR-CFSAN monthly meeting

When: Thursday, January 12, 2017 2:00 PM-3:00 PM (UTC-05:00) Eastern Time (US & Canada).

Where: phone

-- Do not delete or change any of the following text. --

You are the host for this Personal Conference meeting.

Meeting Number: (b) (6)

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Cisco Unified MeetingPlace profile number for meeting host: (b) (6)

FDARichMedia@fda.hhs.gov

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From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#); [Jennifer S. Taylor](#)
Subject: Re: NCNPR-CFSAN monthly meeting
Date: Tuesday, January 24, 2017 9:02:19 PM

Should work. Please give a number to call
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Tuesday, January 24, 2017 at 6:14 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: Re: NCNPR-CFSAN monthly meeting

Friday the 3rd works for me. Would 11 am be OK? Thanks.

From: Ikhlas Khan <ikhlan@olemiss.edu>
Date: January 24, 2017 at 5:51:56 PM EST
To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: Re: NCNPR-CFSAN monthly meeting

Sounds good, IS next Friday Feb 3rd morning will work for?
ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Tuesday, January 24, 2017 at 4:06 PM
To: ikhlas <ikhlan@olemiss.edu>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: RE: NCNPR-CFSAN monthly meeting

Hello,

I think that next week would be good for us to talk. can you please send me a status of the various projects and assays that you have available for cosmetics research? I have new staff that I wish to assign to working with you, and I want to familiarize them with the previous work, as well as the ongoing work and what we have discussed as planned future projects. So next week would be a good time to talk. thank you.

Regards,
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Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road

Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Tuesday, January 24, 2017 4:01 PM

To: Sadrieh, Nakissa

Cc: AMAR GOPAL CHITTIBOYINA

Subject: Re: NCNPR-CFSAN monthly meeting

Hi Nakissa

I know you are busy but I think we should talk about the future work whenever you have time
IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Tuesday, January 10, 2017 at 10:48 AM

To: ikhlas <ikhlan@olemiss.edu>, Cara Welch <Cara.Welch@fda.hhs.gov>, "Swift, Sibyl" <Sibyl.Swift@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Eric Olson <Eric.Olson@fda.hhs.gov>

Cc: JT <jnnfrtyl@olemiss.edu>

Subject: RE: NCNPR-CFSAN monthly meeting

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Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Tuesday, January 10, 2017 11:06 AM

To: Welch, Cara; Swift, Sibyl; Sadrieh, Nakissa; AMAR GOPAL CHITTIBOYINA; Olson, Eric

Cc: Jennifer S. Taylor

Subject: Re: NCNPR-CFSAN monthly meeting

Nakissa, let us know if you want to meet or we can call some other time

ik

From: Cara Welch <Cara.Welch@fda.hhs.gov>

Date: Tuesday, January 10, 2017 at 7:39 AM

To: "Swift, Sibyl" <Sibyl.Swift@fda.hhs.gov>, "Sadrieh, Nakissa"

<Nakissa.Sadrieh@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Eric

Olson <Eric.Olson@fda.hhs.gov>, ikhlas <ikhlan@olemiss.edu>

Cc: JT <jnnfrtyl@olemiss.edu>

Subject: RE: NCNPR-CFSAN monthly meeting

ODSP needs to cancel this month's meeting, Thursday, Jan 12 – OCAC or NCNPR, do you want to keep the meeting amongst yourselves?

Cara

-----Original Appointment-----

From: Welch, Cara

Sent: Thursday, October 13, 2016 3:27 PM

To: Welch, Cara; Swift, Sibyl; Sadrieh, Nakissa; Amar Chittiboyina (amar@olemiss.edu); Olson, Eric; ikhlan@olemiss.edu

Cc: Jennifer S. Taylor

Subject: NCNPR-CFSAN monthly meeting

When: Thursday, January 12, 2017 2:00 PM-3:00 PM (UTC-05:00) Eastern Time (US & Canada).

Where: phone

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From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: NCNPR-CFSAN monthly meeting
Date: Tuesday, January 24, 2017 5:51:56 PM

Sounds good, IS next Friday Feb 3rd morning will work for?
ik

From: "Sadrieh, Nakissa"
Date: Tuesday, January 24, 2017 at 4:06 PM
To: ikhlas
Cc: AMAR GOPAL CHITTIBOYINA
Subject: RE: NCNPR-CFSAN monthly meeting

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Tel: 240-402-2194

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]
Sent: Tuesday, January 24, 2017 4:01 PM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA
Subject: Re: NCNPR-CFSAN monthly meeting
Hi Nakissa

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IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Tuesday, January 10, 2017 at 10:48 AM
To: ikhlas <ikhlan@olemiss.edu>, Cara Welch <Cara.Welch@fda.hhs.gov>, "Swift, Sibyl" <Sibyl.Swift@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Eric Olson <Eric.Olson@fda.hhs.gov>
Cc: JT <jnnfrtyl@olemiss.edu>
Subject: RE: NCNPR-CFSAN monthly meeting

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College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Tuesday, January 10, 2017 11:06 AM
To: Welch, Cara; Swift, Sibyl; Sadrieh, Nakissa; AMAR GOPAL CHITTIBOYINA; Olson, Eric
Cc: Jennifer S. Taylor
Subject: Re: NCNPR-CFSAN monthly meeting
Nakissa, let us know if you want to me on or we can call some other time
ik

From: Cara Welch <Cara.Welch@fda.hhs.gov>
Date: Tuesday, January 10, 2017 at 7:39 AM
To: "Swift, Sibyl" <Sibyl.Swift@fda.hhs.gov>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Eric Olson <Eric.Olson@fda.hhs.gov>, ikhlas <ikhlan@olemiss.edu>
Cc: JT <jnnfrtyl@olemiss.edu>
Subject: RE: NCNPR-CFSAN monthly meeting
ODSP needs to cancel this month's meeting, Thursday, Jan 12 – OCAC or NCNPR, do you want to keep the meeting amongst yourselves?

Cara

-----Original Appointment-----

From: Welch, Cara
Sent: Thursday, October 13, 2016 3:27 PM
To: Welch, Cara; Swift, Sibyl; Sadrieh, Nakissa; Amar Chittiboyina (amar@olemiss.edu); Olson, Eric; ikhlan@olemiss.edu
Cc: Jennifer S. Taylor
Subject: NCNPR-CFSAN monthly meeting
When: Thursday, January 12, 2017 2:00 PM-3:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: phone

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From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: NCNPR-CFSAN monthly meeting
Date: Tuesday, January 24, 2017 4:01:40 PM

Hi Nakissa

I know you are busy but I think we should talk about the future work whenever you have time
IK

From: "Sadrieh, Nakissa"

Date: Tuesday, January 10, 2017 at 10:48 AM

To: ikhlas , Cara Welch , "Swift, Sibyl" , AMAR GOPAL CHITTIBOYINA , Eric Olson

Cc: JT

Subject: RE: NCNPR-CFSAN monthly meeting

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4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [mailto:ikhlan@olemiss.edu]

Sent: Tuesday, January 10, 2017 11:06 AM

To: Welch, Cara; Swift, Sibyl; Sadrieh, Nakissa; AMAR GOPAL CHITTIBOYINA; Olson, Eric

Cc: Jennifer S. Taylor

Subject: Re: NCNPR-CFSAN monthly meeting

Nakissa, let us know if you want to me on or we can call some other time

ik

From: Cara Welch <Cara.Welch@fda.hhs.gov>

Date: Tuesday, January 10, 2017 at 7:39 AM

To: "Swift, Sibyl" <Sibyl.Swift@fda.hhs.gov>, "Sadrieh, Nakissa"

<Nakissa.Sadrieh@fda.hhs.gov>, AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>, Eric Olson <Eric.Olson@fda.hhs.gov>, ikhlas <ikhlan@olemiss.edu>

Cc: JT <jnnfrtyl@olemiss.edu>

Subject: RE: NCNPR-CFSAN monthly meeting

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Cara

-----Original Appointment-----

From: Welch, Cara

Sent: Thursday, October 13, 2016 3:27 PM

To: Welch, Cara; Swift, Sibyl; Sadrieh, Nakissa; Amar Chittiboyina (amar@olemiss.edu); Olson, Eric; ikh@olemiss.edu

Cc: Jennifer S. Taylor

Subject: NCNPR-CFSAN monthly meeting

When: Thursday, January 12, 2017 2:00 PM-3:00 PM (UTC-05:00) Eastern Time (US & Canada).

Where: phone

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From: [Ikhlas Khan](#)
To: [Welch, Cara](#); [Swift, Sibyl](#); [Sadrieh, Nakissa](#); [AMAR GOPAL CHITTIBOYINA](#); [Olson, Eric](#)
Cc: [Jennifer S. Taylor](#)
Subject: Re: NCNPR-CFSAN monthly meeting
Date: Tuesday, January 10, 2017 11:06:50 AM

Nakissa, let us know if you want to me on or we can call some other time
ik

From: Cara Welch

Date: Tuesday, January 10, 2017 at 7:39 AM

To: "Swift, Sibyl" , "Sadrieh, Nakissa" , AMAR GOPAL CHITTIBOYINA , Eric Olson , ikhlas

Cc: JT

Subject: RE: NCNPR-CFSAN monthly meeting

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Cara

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From: Welch, Cara

Sent: Thursday, October 13, 2016 3:27 PM

To: Welch, Cara; Swift, Sibyl; Sadrieh, Nakissa; Amar Chittiboyina (amar@olemiss.edu); Olson, Eric; ikhan@olemiss.edu

Cc: Jennifer S. Taylor

Subject: NCNPR-CFSAN monthly meeting

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From: [Ikhlas Khan](#)
To: [Welch, Cara](#); [Swift, Sibyl](#); [Sadrieh, Nakissa](#); [AMAR GOPAL CHITTIBOYINA](#); [Olson, Eric](#)
Cc: [Jennifer S. Taylor](#)
Subject: Re: NCNPR-CFSAN monthly meeting
Date: Thursday, May 11, 2017 12:23:56 PM

You are the boss. We are fine if you cancel
ik

From: Cara Welch
Date: Thursday, May 11, 2017 at 11:20 AM
To: "Swift, Sibyl" , "Sadrieh, Nakissa" , AMAR GOPAL CHITTIBOYINA , Eric Olson , ikhlas
Cc: Jennifer
Subject: RE: NCNPR-CFSAN monthly meeting
Any objection to cancelling this month's meeting?
-----Original Appointment-----
From: Welch, Cara
Sent: Thursday, October 13, 2016 3:27 PM
To: Welch, Cara; Swift, Sibyl; Sadrieh, Nakissa; Amar Chittiboyina (amar@olemiss.edu); Olson, Eric; ikhan@olemiss.edu
Cc: Jennifer S. Taylor
Subject: NCNPR-CFSAN monthly meeting
When: Thursday, May 11, 2017 2:00 PM-3:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: phone

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From: [Ikhlas Khan](#)
To: [Katz, Linda](#)
Cc: [Welch, Cara](#); [Sadrieh, Nakissa](#)
Subject: Re: next year
Date: Monday, March 14, 2016 10:13:10 AM

Dear Linda

Thanks for quick and positive response. Yes, we will be discussing with Nakissa for upcoming projects.

Appreciate your help

IK

From: "Katz, Linda" <Linda.Katz@fda.hhs.gov>
Date: Monday, March 14, 2016 at 8:06 AM
To: Ikhlas Khan <ikhan@olemiss.edu>
Cc: Cara Welch <Cara.Welch@fda.hhs.gov>, "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Subject: RE: next year

Ikhlas,

Thanks for your email follow-up. We are working on our budget allocations and it looks like this year we may be able to increase our support, especially since Nakissa has some additional projects that she would like to discuss with you when she visits in April. I should be able to provide you with an approximate amount later this month after we discuss our proposed allocations with our Office of Management.

Linda

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]
Sent: Monday, March 14, 2016 9:59 AM
To: Katz, Linda
Cc: Welch, Cara
Subject: next year

Dear Linda

Hope you are doing fine. Under the guidance of Stan and Nakissa project is developing well. We are setting up new screens, it will be a very good joint effort with your division and the center.

As you probably know, this year we are going for 5 year cycle renewal. Last year we had some budget issue but hope to have more resources in future for expanded work and I know you will do your best.

Please keep us in mind when you work on budget for next year.

Conference is coming up soon and we do have session on cosmetics. This will also give opportunity for Nakisha to visit the labs.

Thanks for all your support and if we can be any help please let us know.

ik

From: [AMAR GOPAL CHITTIBOYINA](#)
To: [Moghaddam, Sarvin](#)
Cc: [Vukmanovic, Stanislav](#); [Sadrieh, Nakissa](#); [Ikhlas Khan](#)
Subject: RE: products with Aloe ingredient
Date: Thursday, August 03, 2017 4:28:24 PM

Thank you and will be very helpful.

From: Moghaddam, Sarvin [mailto:Sarvin.Moghaddam@fda.hhs.gov]

Sent: Thursday, August 3, 2017 3:24 PM

To: AMAR GOPAL CHITTIBOYINA

Cc: Vukmanovic, Stanislav ; Sadrieh, Nakissa

Subject: products with Aloe ingredient

Hi Amar,

Attached please find the products with Aloe ingredients. Please let me know if you need any additional information.

Both files represent the same products in different formats.

Thank you

Sarvin

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: recent research data
Date: Wednesday, October 12, 2016 10:23:39 PM

Dear Nakisha

We are in process to prepare the documents as you asked us to prepare. We will be sending them to you soon. We will try to get you the slides hopefully by Monday.

I will be in DC for COE meeting, would be around around 27th October?

ik

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, October 12, 2016 at 4:13 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Subject: recent research data

Hello Ikhlas,

I hope that you are well. I need to give a presentation in a couple of weeks, on cosmetics research that our office is engaged in. I wanted to include some of the data that your lab has done on the in vitro testing of allergens, for sensitization, using the in vitro assays. Can I please ask you to send me some slides on your work from this past year, where you screened some of the fragrance allergens, using all the in vitro methods available to you? if you would also provide a slide for each of the methods used, to describe the principle of the method, along with a separate narrative for each slide, that would be great. this will also help me, as I prepare to update Linda on all of our research projects from the past year, as this is something that we do at the beginning of the fiscal year.

If you could provide the slides in PowerPoint, that would be great, and the narratives, can be in WORD, and refer to the specific slide, in order to describe the basis or principle of the method used. Will you be able to send me something by next Monday, COB? Thank you.

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

Office of Cosmetics and Colors (OCAC)

Center for Food Safety and Applied Nutrition (CFSAN)

Food and Drug Administration (FDA)

4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Katz, Linda](#)
Cc: [Sadrieh, Nakissa](#); [Welch, Cara](#)
Subject: Re: request
Date: Monday, June 05, 2017 4:41:02 PM

Dear Linda

Thanks for quick response. We appreciate your continuous support for the program. We will discuss with Nakissa regarding the work and future plans.

Thanks

IK

On 6/5/17, 3:00 PM, "Katz, Linda" <Linda.Katz@fda.hhs.gov> wrote:

Ikhlas,

Nakissa has been keeping me informed and we are planning on continuing our support at \$250,000.

Linda

Linda M. Katz, M.D., M.P.H.
Director, Office of Cosmetics and Colors
Acting Chief Medical Officer Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive, HFS-100
College Park, Maryland 20740

240-402-1130 (phone)

301-436-2976 (fax)

linda.katz@fda.hhs.gov

-----Original Message-----

From: Ikhlas Khan [<mailto:ikhan@olemiss.edu>]

Sent: Monday, June 05, 2017 3:51 PM

To: Katz, Linda

Cc: Sadrieh, Nakissa; Welch, Cara

Subject: request

Dear Linda

Hope you are doing well. I am sure you are aware of the progress we have made with the help and guidance from Nakissa. Its time again to ask you for your support. I hope to continue the program at the same level.

Thanks for your Support

IK

Ikhlas A. Khan, Ph.D, D. Litt (Hon. Causa) Director, NCNPR

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [Jennifer S. Taylor](#)
Subject: Re: sorry, i was in a meeting
Date: Thursday, October 08, 2015 4:26:34 PM

Great talking to you. Here is the website for conference.
Oxfordicsb.org and if you need any assistance or further information, Jennifer will help you.

We are open to provide support for conference if needed.

Looking forward to working with you

IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Thursday, Oct 8, 2015 at 3:06 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: sorry, i was in a meeting

What is your phone number?

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: sorry, i was in a meeting
Date: Thursday, October 08, 2015 4:11:32 PM

Office 662 915 7821

Cell# (b) (6)

Call me when you are done

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>

Date: Thursday, October 8, 2015 at 3:06 PM

To: Ikhlas Khan <ikhlan@olemiss.edu>

Subject: sorry, i was in a meeting

What is your phone number?

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: sorry, i was in a meeting
Date: Saturday, October 24, 2015 2:36:34 AM

Dear Nakissa

I am sure you did not forget but wanted to send a reminder for ICSB session that we discussed.

We can discuss when you are ready. I should be back soon

IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Thursday, October 8, 2015 at 3:06 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Subject: sorry, i was in a meeting

What is your phone number?

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: Thanks
Date: Wednesday, July 19, 2017 9:51:54 AM

Dear Nakissa

Hope you are doing fine. Amar is in touch and keeping you up to date but if we have time we can arrange a call to discuss future plans.

We have been contacted by Linda Loretz, personal care products council, they have their annual Symposium in October at Virginia. They would like to have safety of botanicals. I assume you know them well. What is your recommendation, I think we should present what we are doing here but needed your advise.

Thanks

Ik

From: "Sadrieh, Nakissa"

Date: Wednesday, June 14, 2017 at 7:34 PM

To: Ikhlas Khan

Subject: Re: Thanks

Hi Ikhlas,

I am sorry for not responding to your email sooner. I have spoken with Linda and asked her to send U Miss any additional funds, if there are any more funds available. We are happy with the work that we are doing with your lab and we hope to continue working with you. Our budget situation may be a little shaky with the new administration and everyone is being cautious. I have asked if an additional \$30-50K could be sent to your lab, if we have the funds. I will let you know if funds become available. Thank you.

Nakissa.

From: Ikhlas Khan

Date: June 5, 2017 at 4:50:13 PM EDT

To: Sadrieh, Nakissa

Subject: Thanks

Hi Nakissa

Hope you are doing fine. I got the response from Linda and this would not have been possible without your involvement. It took sometime but I think now you feel comfortable with what we have here and continuing this effort is crucial. I am happy to hear that it's not drastic cut in budget but program like us it is something. Is there any way we can get same amount as last year.

I am asking on personal basis, you can tell me that be happy what we got and I am

If you have few minutes we can chat my cell (b) (6)

Thanks

IK

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Subject: Re: Thanks
Date: Thursday, June 15, 2017 4:59:43 AM

Thanks. Appreciate so much

Sent from my iPhone

On Jun 15, 2017, at 8:34 AM, Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov> wrote:

Hi Ikhlas,

I am sorry for not responding to your email sooner. I have spoken with Linda and asked her to send U Miss any additional funds, if there are any more funds available. We are happy with the work that we are doing with your lab and we hope to continue working with you. Our budget situation may be a little shaky with the new administration and everyone is being cautious. I have asked if an additional \$30-50K could be sent to your lab, if we have the funds. I will let you know if funds become available. Thank you.

Nakissa.

From: Ikhlas Khan <ikhlan@olemiss.edu>
Date: June 5, 2017 at 4:50:13 PM EDT
To: Sadrieh, Nakissa <Nakissa.Sadrieh@fda.hhs.gov>
Subject: Thanks

Hi Nakissa

Hope you are doing fine. I got the response from Linda and this would not have been possible without your involvement. It took sometime but I think now you feel comfortable with what we have here and continuing this effort is crucial. I am happy to hear that it's not drastic cut in budget but program like us it is something. Is there any way we can get same amount as last year.

I am asking on personal basis, you can tell me that be happy what we got and I am

If you have few minutes we can chat my cell (b) (6)

Thanks

IK

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: Thanks
Date: Wednesday, July 19, 2017 2:48:21 PM

Please some time, its important topic to discuss
ik

From: "Sadrieh, Nakissa"
Date: Wednesday, July 19, 2017 at 9:34 AM
To: Ikhlas Khan
Cc: AMAR GOPAL CHITTIBOYINA
Subject: Re: Thanks

Hello,

I also think that we should discuss this,

Since the work is being done for FDA, and we may want to be careful about how we present the data to the public. Thanks.

From: Ikhlas Khan
Date: July 19, 2017 at 9:51:53 AM EDT
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA
Subject: Re: Thanks

Dear Nakissa

Hope you are doing fine. Amar is in touch and keeping you up to date but if we have time we can arrange a call to discuss future plans.

We have been contacted by Linda Loretz, personal care products council, they have their annual Symposium in October at Virginia. They would like to have safety of botanicals. I assume you know them well. What is your recommendation, I think we should present what we are doing here but needed your advise.

Thanks

Ik

From: "Sadrieh, Nakissa"
Date: Wednesday, June 14, 2017 at 7:34 PM
To: Ikhlas Khan
Subject: Re: Thanks

Hi Ikhlas,

I am sorry for not responding to your email sooner. I have spoken with Linda and asked her to send U Miss any additional funds, if there are any more funds available. We are happy with the work that we are doing with your lab and we hope to continue working with you. Our budget situation may be a little shaky with the new administration and everyone is being cautious. I have asked if an additional \$30-50K could be sent to your lab, if we have the funds. I will let you know if funds become available. Thank you.

Nakissa.

From: Ikhlas Khan

Date: June 5, 2017 at 4:50:13 PM EDT

To: Sadrieh, Nakissa

Subject: Thanks

Hi Nakissa

Hope you are doing fine. I got the response from Linda and this would not have been possible without your involvement. It took sometime but I think now you feel comfortable with what we have here and continuing this effort is crucial. I am happy to hear that it's not drastic cut in budget but program like us it is something. Is there any way we can get same amount as last year.

I am asking on personal basis, you can tell me that be happy what we got and I am

If you have few minutes we can chat my cell# (b) (6)

Thanks

IK

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: Visit
Date: Wednesday, June 08, 2016 3:57:46 PM

Hi Nakissa

Yes, that will be fine. In the mean time we can have a call to make sure everything is progressing well or you prefer to discuss during your visit in September
IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, June 8, 2016 at 2:54 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: RE: Visit

Hello,

I am sorry for not getting back to you sooner. I would like to come for a visit, but at this point, I might come over in September, if that is OK with you. it is still within the fiscal year, and the summer will have passed. Please let me know, so that I may look into the travel here. thanks.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Wednesday, June 08, 2016 3:42 PM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA
Subject: Visit
Hi Nakissa

Hope you are doing fine. During your ICSB visit you mentioned that you would like to visit us to discuss science. Please let me know when you will have time to visit us
IK

From: [Ikhlas Khan](#)
To: [Sadrieh, Nakissa](#)
Cc: [AMAR GOPAL CHITTIBOYINA](#)
Subject: Re: Visit
Date: Wednesday, June 08, 2016 4:03:51 PM

Ok, Amar will try to get summary together and send it to you before we setup a call.
Please suggest your time for September visit
IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, June 8, 2016 at 2:59 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: RE: Visit

I think that we should have a call to talk about how the studies are progressing, and if additional work needs to be considered. This way, when we see each other, we can have data to look at and make further plans. Do you have any data that you can send to us at this point, to review, before we talk? Thanks.

Regards,
Nakissa Sadrieh, Ph.D.
Director, Cosmetics Division
Office of Cosmetics and Colors (OCAC)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration (FDA)
4300 River Road
Room 1034 (HFS-125)
College Park, MD 20740
Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]
Sent: Wednesday, June 08, 2016 3:58 PM
To: Sadrieh, Nakissa
Cc: AMAR GOPAL CHITTIBOYINA
Subject: Re: Visit
Hi Nakissa

Yes, that will be fine. In the mean time we can have a call to make sure everything is progressing well or you prefer to discuss during your visit in September
IK

From: "Sadrieh, Nakissa" <Nakissa.Sadrieh@fda.hhs.gov>
Date: Wednesday, June 8, 2016 at 2:54 PM
To: Ikhlas Khan <ikhlan@olemiss.edu>
Cc: AMAR GOPAL CHITTIBOYINA <amar@olemiss.edu>
Subject: RE: Visit

Hello,

I am sorry for not getting back to you sooner. I would like to come for a visit, but at this point, I might come over in September, if that is OK with you. it is still within the fiscal year, and the summer

will have passed. Please let me know, so that I may look into the travel here. thanks.

Regards,

Nakissa Sadrieh, Ph.D.

Director, Cosmetics Division

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4300 River Road

Room 1034 (HFS-125)

College Park, MD 20740

Tel: 240-402-2194

From: Ikhlas Khan [<mailto:ikhlan@olemiss.edu>]

Sent: Wednesday, June 08, 2016 3:42 PM

To: Sadrieh, Nakissa

Cc: AMAR GOPAL CHITTIBOYINA

Subject: Visit

Hi Nakissa

Hope you are doing fine. During your ICSB visit you mentioned that you would like to visit us to discuss science. Please let me know when you will have time to visit us

IK

A. COVER PAGE

Project Title: Science based authentication of botanical ingredients	
Grant Number: 5U01FD004246-07	Project/Grant Period: 09/15/2011 - 08/31/2021
Reporting Period: 09/05/2016 - 08/31/2017	Requested Budget Period: 09/01/2017 - 08/31/2018
Report Term Frequency: Annual	Date Submitted: 06/30/2017
Program Director/Principal Investigator Information: IKHLAS AHMAD KHAN , PHD Phone number: (662) 915-7821 Email: ikhan@olemiss.edu	Recipient Organization: UNIVERSITY OF MISSISSIPPI UNIVERSITY OF MISSISSIPPI 100 BARR HALL UNIVERSITY, MS 386770907 DUNS: 067713560 EIN: 1646001159A1 RECIPIENT ID:
Change of Contact PD/PI: N/A	
Administrative Official: JASON G HALE 100 Barr Hall University, MS 38677 Phone number: 6629157482 Email: research@olemiss.edu	Signing Official: JASON G HALE 100 Barr Hall University, MS 38677 Phone number: 6629157482 Email: research@olemiss.edu
Human Subjects: No	Vertebrate Animals: No
hESC: No	Inventions/Patents: No

B. ACCOMPLISHMENTS**B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?**

In 2001, a cooperative research agreement was established between the University of Mississippi's National Center for Natural Products Research (NCNPR) and the Center for Food Safety and Applied Nutrition (CFSAN), U.S. Food and Drug Administration (FDA), to address critical research issues related to the potential health impact of the use of botanical dietary ingredients (BDIs) by U.S. consumers. This coincides with FDA's strategy to establish "Centers of Excellence" for collaborative research relationships that support the FDA's scientific mission. In particular, this agreement outlines collaborative efforts that are aimed at developing and disseminating authenticated botanical reference materials, methods, and information that will assist in developing a scientific base and the available tools for authentication, analysis, and safety evaluation of botanical ingredients in order to provide information that can be used by the FDA to develop, implement and modify regulatory guidance. The intention of this proposed continuation of the existing, fifteen year, ongoing cooperative research agreement is to further these evaluations for existing and future botanical dietary ingredients that are deemed a priority with regard to public safety. In order to accomplish this task the NCNPR has established the following specific aims to assist in addressing these research needs in order to support the mission of the FDA:

1. Assist in the identification and development of a list of dietary herbal supplements and botanical ingredients, based on safety concerns, trends, and knowledge of botanicals being marketed in the U.S., to prioritize for further research.
2. Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to FDA, for the assessment of their fingerprinting, adulteration, safety and toxicity.
3. Exchange technical and scientific information, analytical methods, and reference materials with FDA scientists and other stakeholders.
4. Collaborate with FDA scientists in research areas of mutual interest.
5. Coordinate scientific workshops and conferences on BDI-related topics of public health relevance to address high priority science and public awareness of emerging problems associated with botanicals.

These efforts will include the following major components: the identification of BDI's that could present potential safety issues to the consumer; implementing botanical/morphological identification techniques and genetic profiling with help of DNA-barcoding in order to authenticate plant material(s); basic preliminary biological evaluations of crude extracts, column fractions and pure compounds isolated from authenticated materials; chemical fingerprinting techniques including the isolation and identification of marker compounds and putative active constituents; development of new reference materials and techniques; assessment of undesired psychoactive and hepatotoxic potential of extracts, fractions and pure compounds; and stability and safety of volatile organic materials in cosmetics and other personal care products. These data will be analyzed, organized and reported in a 'research dossier' for FDA investigators and where appropriate, published in peer-reviewed literature. This body of information and the developed methods can be used for cGMP training, monitoring inspections, and regulatory aspects of CFSAN's mission on botanical dietary ingredients. Lastly the NCNPR has culminated these efforts with the hosting of an annual international conference on the science of botanicals (ICSB) in order to review, discuss and disseminate information regarding the issues related to authentication, quality and safety of botanicals. The outcome from this work has significantly augmented the science base and available tools for authentication, analysis, and safety evaluation of botanical ingredients in supplements. Most importantly the 'ICSB platform' has afforded a venue for dialog with industry and trade associations, and with international governments that can be used by the FDA to further develop regulatory guidelines for products that are adulterated (e.g., that the product is unsafe) or mis-branded. It is the intention of this proposal to extend the current cooperative research agreement in order to further these evaluations for existing and future botanical dietary ingredients that are deemed a priority public safety concern to the FDA.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Progress Report 2016-2017_Final.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

To address the complex issues associated with botanical dietary supplements and in continuation of our on-going cooperative agreement with the Center for Food Safety and Applied Nutrition, the National Center for Natural Products Research has established the following specific aims to facilitate the research needs of the FDA. The following goals are specifically designed to address the issues with botanical identity, authenticity, quality and safety.

1. Assist in the identification and development of a list of botanical ingredients to prioritize for further research taking account of safety concerns, trends, and knowledge of botanicals being marketed in the U.S.
2. Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to the FDA, for the assessment of their fingerprinting, adulteration and safety.
3. Exchange technical and scientific information, analytical methods and reference material with FDA scientists and other stakeholders.
4. Collaborate with FDA scientists in research areas of mutual interest.
5. Coordinate scientific workshops and conferences on botanical ingredient-related topics of public health relevance to address high priority science and public awareness of emerging problems associated with botanicals.

Progress Report Summary (2016-2017)

A. Specific Aims

To address the complex issues associated with botanical dietary supplements and in continuation of our on-going cooperative agreement with the Center for Food Safety and Applied Nutrition, the National Center for Natural Products Research has established the following specific aims to facilitate the research needs of the FDA. The following goals are specifically designed to address the issues with botanical identity, authenticity, quality and safety.

1. Assist in the identification and development of a list of botanical ingredients to prioritize for further research taking account of safety concerns, trends, and knowledge of botanicals being marketed in the U.S.
2. Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to the FDA, for the assessment of their fingerprinting, adulteration and safety.
3. Exchange technical and scientific information, analytical methods and reference material with FDA scientists and other stakeholders.
4. Collaborate with FDA scientists in research areas of mutual interest.
5. Coordinate scientific workshops and conferences on botanical ingredient-related topics of public health relevance to address high priority science and public awareness of emerging problems associated with botanicals.

B. Studies and Results:

Aim 1: Assist in the identification and development of a list of botanical ingredients to prioritize for further research taking account of safety concerns, trends, and knowledge of botanicals being marketed in the U.S.

Since the inception of the cooperative agreement between the NCNPR and the FDA in 2001, there have been numerous occasions for interactions between the two organizations. These have included (yearly) formal site visits by the FDA delegates (Dr. Mickey Parish, Dr. Eric Olson, Mr. Tave, Dr. Cara Welch and Dr. Sibyl Swift), interactions during the annual International Conference on the Science of Botanicals (ICSB), several monthly conference calls and numerous email exchanges. In addition to these interactions, over the past year the NCNPR has hosted three training sessions with the Office of Regulatory Affairs (ORA) to provide hands-on training of FDA inspectors for cGMP compliance issues associated with botanical ingredients (FD340). Under this aim, 97 inspectors were trained during 2016-2017. These training sessions have provided an opportunity for Drs. Welch and Swift, to visit the NCNPR and stay abreast of the Center's ongoing research efforts. As always, researchers at the NCNPR are receptive to areas of study that are deemed necessary by the ODSP/CFSAN for this cooperative agreement.

As in the past, these areas of interest may be modified and added to as the Steering Committee recommends, based on emerging safety concerns or preliminary data developed. Representative methodological approaches are presented here for select botanicals and compound classes of interest. Details of the extractions, isolations, purifications and analyses will, of course, vary depending on the plants selected, the required levels of detection, the compounds' chemical characteristics and other factors.

Aim 2: Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to the FDA, for the assessment of their fingerprinting adulteration and safety.

The availability of authenticated reference materials for botanicals is an ideal first step in the development of analytical methodologies for the identification and quantitative analysis of botanical product(s). The NCNPR has been very successful in placing formal agreements with several international academic and governmental institutions from Southeast Asia, China, Europe South Africa, Central/South America. In addition to an on-going Memorandum of Understanding (MOU) with the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Brazil and the Natural Products Utilization Research Unit (NPURU), USDA located in Oxford

Mississippi, the NCNPR signed MOUs with Indonesia and Thailand for exchanging the reference botanicals. Moreover, the NCNPR has cultivated a productive relationship with industry partners such as McCormick & Co. in order to obtain relevant authenticated herbs and spices for our research needs. With this mechanism, NCNPR has acquired several varieties of black pepper from seven countries to study the chemovariation of phytochemicals present in essential oil of black pepper. As a part of collaboration, several samples of *Sceletium tortuosum* and rooibos tea samples were provided by Tshwane University of Technology, Pretoria to exchange the traditional practices based on botanicals endemic to South Africa and other related plant materials of interest to the FDA.

From these and other collaborative relationships, the NCNPR has been able to acquire several plant samples and herbal extracts. There is a continuous effort to acquire authentic plant samples as well for standardization purposes in addition to assuring that the developed identity testing techniques for this project are applicable for finished products in various formularies. All the plants and samples collected for this program have been assigned an inventory number and are cataloged in the NCNPR repository and voucher specimens of authentic samples are also maintained at the Thomas M. Pullen Herbarium in the Department of Biology at the University of Mississippi. Considerable efforts were made to achieve a searchable repository database with images and a dedicated display room for various botanicals.

In addition to the repository, the Maynard W. Quimby Medicinal Plant Garden maintains more than 300 species for selected growing in agriculture fields, greenhouse or shade houses and has developed significant collaborations with seed banks from 40 institutes around the world in order to exchange medicinally important germplasms. This seed bank effort has yielded a collection of over 1,730 species to date. In addition, so far, the garden personnel added 500 plant vouchers and are collecting samples for a DNA tissue bank for genetic barcode purposes. The Medicinal Plant Garden is also registered under the Botanic Gardens Conservation International and is a member of the American Association of Botanical Gardens and Arboreta and International Plant Exchange Network (IPEN). Overall, this garden provides not only an invaluable resource for propagating and sourcing botanicals of interest but also provides a training facility for FDA/ORA courses on identification of botanicals of interest.



Figure 1. Demonstrating the proper authentication techniques for the identification of Botanical Dietary Supplements (BDS) as a part of Course FD340.

Macroscopic techniques are routinely used in our labs to discriminate between the desired plant species or plant part, and morphologically similar, yet distinguishable, species or parts that could occur as potential adulterants. Microscopic techniques are performed in our labs to assure authenticity or detect adulteration in ground plant samples where macroscopic characteristics are difficult to observe. For example, bay leaf is a popular household spice used in food flavoring and the 'true' bay leaf is derived only from *Laurus nobilis*, which is native to the Mediterranean region. However, leaves of several other plants, including *Cinnamomum tamala* (Indian bay leaf), *Litsea glaucescens* (Mexican bay leaf), *Pimenta racemosa* (West Indian bay leaf), *Syzygium polyanthum* (Indonesian bay leaf) and *Umbellularia californica* (Californian bay leaf), are also often sold as 'bay leaves' and are commonly used in commerce. A detailed comparative study¹ was conducted and leaf morphological, anatomical features of *L. nobilis* and its common surrogates were studied for correct identification purposes. The odor and flavor of these leaves are, however, not the same as the true bay leaf, and for that reason, they should not be used in cooking as a substitute for *L. nobilis*. Some of the bay leaf substitutes such as *Umbellularia* spices can also cause headache and other adverse effects.

In addition to classical taxonomic identification techniques, we are also implementing DNA fingerprinting studies to authenticate and validate reference plant materials. To overcome the limitations of full-length 'DNA barcodes', development of 'DNA mini barcodes', was undertaken in our labs. The advantages of mini barcodes are easy retrieval of DNA markers even from processed dietary materials due to their small amplicon length, and the ability to distinguish closely related species because of the genus/ species specificity. DNA mini barcodes based on two genomic regions: ITS (nuclear) and psbA-trnH (chloroplast), were developed and

utilized for the identification of previously mentioned six bay leaves. Currently, we are evaluating the applicability of 'terpene synthases as mini barcodes' for botanical identification purposes. The untested idea is to develop a set of plant universal oligos/primers that can bind to most terpene synthases and amplify a portion of the gene. The resulting fragments could be sequenced and can be applied for plant identification. We are working closely with Dr. Sara Handy at CFSAN on implementing terpene synthases as mini barcodes for select botanicals.

Isolation of reference compounds: Often isolation of standard compounds is required to develop analytical methods, quantify individual compounds in extracts and to establish analytical fingerprints in support of authenticity, quality, safety and toxicity studies. A number of compounds from *Sceletium tortuosum*, *Aframomum melegueta*, Red Yeast Rice,² *Kigelia africana*, *Epimedium grandiflorum* and *Cnicus benedictus*³ have been isolated and published in various scientific journals. The isolation and characterization of several secondary metabolites from *Sutherlandia frutescens*, *Bulbine natalensis*, *Fadogia agrestis*, *Acacia rigidula*, *Moringa oleifera*, *Tinospora* species, *Mimosa pigra*, and *Urtica dioica* are on-going and the results will be reported in due course. It is through these continued efforts that the NCNPR scientists have isolated several novel compounds and known analogs for analytical fingerprinting development for authentication purposes as well as pharmacological, safety analysis such as herb-drug interactions.

Synthesis and procurement of compounds of interest: In certain situations, synthesis of reference compounds was also undertaken at NCNPR, especially when isolation of marker compounds was laborious and time-consuming. Several compounds of interest, dimethylhexylamine, bis(dimethylhexyl)amine, *racemic*-aegeline, (+)- and (-)-aegeline were synthesized from commercially available raw materials on a bulk scale. In addition to large scale synthesis, several single enantiomers, such as *S*-aegeline, were synthesized for the development of analytical methods to understand the origin (synthetic/natural) of compounds of interest and their herb-drug interaction potential of *A. marmelos*.^{4, 5}

Analytical method development and botanical fingerprint profiling: In the past year, we have focused on developing several analytical methods for the determination of the authenticity of BDS's of interest. These studies include the development of various analytical methods such as UPLC (UV, ELSD and MS), Supercritical Fluid Chromatography (SFC) and GC-MS. A concurrent SFC method was developed and applied to analysis of terpene lactones and ginkgolic acids in *G. biloba* extracts and various ginkgo-based dietary supplements.⁶ In combination with chemometric modeling software, an ultrahigh-performance LC QToF mass spectrometric method was used for generating comprehensive fingerprints of three distinctive botanicals, namely, *Hoodia*, *Terminalia* and chamomile.⁷ Method development, characterization, chemical profiling and MS/MS analysis of *Asimina triloba* (Paw Paw) *Calea zacatechichi*, *Huperzia serrata*, *Fadogia agrestis* and several other botanicals are on-going in our labs. In addition to method development for chemical finger-printing needs, a VALLME (vortex-assisted liquid-liquid microextraction) method combined with UHPLC-ELSD for the determination of four cucurbitane triterpenoids in different bitter melon drinks was developed and compared with traditional extraction methods.⁸ Moreover, we are implementing Waters UNIFI platform to merge LC and high performance MS data into a single solution for determination of marker compounds and adulterants from various dietary supplements. As a proof-of-concept, UHPLC-UV-MS/MS with UNIFI platform was developed and several dietary supplements based on *Eleutherococcus senticosus* (Ci-Wu-Jia tea) were studied. The presence of EGCG, ECG, EGC, theobromine, and caffeine indicated that several Ci-Wu-Jia teas were adulterated with green tea extracts. It is our main intention to apply UNIFI platform for the establishment of natural product libraries and chemical profiles for *Huperzia serrata*, *Calea zacatechichi*, and *Hypoxis hemerocallidea*.

Several sympathomimetic compounds are continuously introduced in the dietary supplements market and these questionable ephedrine-like, products are specifically targeted to certain consumers with the promise of products designed to suppress appetite or enhance physical and mental performances. Banning of the ephedra alkaloids resulted in the introduction of synephrines and other designer compounds and this unscrupulous trend is burgeoning with new ingredients and new products in the current trendy dietary supplement market. These ingredients are often labeled as 'natural' constituents even though they may originate from un-tested, non-validated botanical sources. Our previous work on phantom compounds, such as DMAA and DMBA, highlights the importance of method development and implications in establishing the origin of the ingredient (natural or synthetic). Analogous to DMAA, DMBA, a new type of dietary supplement, has

recently appeared in the market claiming to contain 2-aminoisoheptane (DMHA) which was touted as a natural ingredient. The natural source of this compound has been cited as fish, cheese, algae, tea as well as numerous plants, viz., *Aconitum kusnezoffii*, *Juglans regia*, *Elytarria acualis*, *Callicarpa formosana*, *Kigelia africana*, *Forsythia suspense*, *Biophytum veldkampii* and finally geranium (*Pelargonium graveolens*). Thus, 2-aminoisoheptane would appear to be almost ubiquitous. In order to establish the quality, quantity and source of DMHA, two GC-MS methods were developed for plant and commercial products analysis. The first method, conventional GC/MS method with a megabore capillary column, was developed for effective chromatographic separation to increase retention and partially diminish tailing effects, and second a chiral method to resolve the enantiomers of the dimethyl amines for more specific identification. The method validation, quantification and chiral results are completed and will be published shortly. Even though the labels claimed the presence of DMHA, only four products actually contained DMHA always in racemic form. None of the studied plant samples contained DMHA (up to 25 ppb level).

Aim 3: Exchange technical and scientific information, analytical methods, and reference material with FDA scientists and other stakeholders.

Dr. Ikhlas Khan, Scientific Director for the project, has been in touch regularly with Mr. Tave [Director, Office of Dietary Supplement Programs (ODSP), CFSAN], Dr. Cara Welch (Senior Advisor, ODSP), Dr. Sibyl Swift (Special Assistant, ODSP), Dr. Linda Katz [Director, Office of Cosmetics and Colors (OCAC), CFSAN] and Dr. Nakissa Sadrieh (CFSAN/OCC, Director – Cosmetics Division) to exchange information on developed methods, reference materials availability, safety evaluations and overall project direction. These meetings and calls are designed to provide these individuals with an update of the NCNPR's overall program and to gain insight as to how this Center can diligently address the needs of the ODSP and OCAC at CFSAN.

Additionally, the NCNPR FDA-COE has also continued to assist the FDA's ORA in training inspectors for cGMP compliance for BDS's (FD340). Researchers at the NCNPR provided three one-day workshops on botanical dietary supplement authentication techniques to 97 trainees and FDA officials on December 7th 2016, March 3rd and May 17th, 2017. The main training course was held in Memphis, Tennessee. As a part of training course a one-day trip to the NCNPR for a combination of lectures and laboratory courses and hands-on training sessions to see what authentication techniques can be implemented for identification of botanical ingredients in dietary supplements. The course covered current techniques utilized to identify botanical materials (microscopy, taxonomy, macroscopy, TLC, HPLC, UPLC, GC, etc.) and was presented by Dr. Khan and colleagues at the NCNPR and included two lab courses on "Analytical Methods, Botanical Authentication and Nomenclature".

Lastly, the research effort initiated by the establishment of this COE has provided several opportunities to leverage the existing cooperative agreement to expand the impact and overall benefit of the existing program. This includes funding from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) /Drug-Induced Liver Injury Network (DILIN) to collect and analyze cases of severe liver injury caused by prescription drugs or over-the-counter drugs, and alternative medicines, such as herbal products or supplements. (PD: Navarro, Victor, Einstein Healthcare Network - Award number 3U01DK083027-09S2). Under this grant, the NCNPR is providing the chemical analysis of various supplement products implicated in liver injury. Additionally, the NCNPR has obtained funding from the USDA/ARS for a grant entitled "Discovery and Development of Natural Product-Based Insect Management Compounds for Medicinal, Veterinary and Urban Concern", award number 58-6066-6-043. The purpose of this grant is to discover and develop new, safer and more effective insect management products derived from natural phytochemical sources.

Aim 4: Collaborate with FDA scientists in research areas of mutual interest.

Skin sensitization – Alternative testing methods: Botanical extracts and single compounds extracted from botanicals are increasingly used in cosmetics, fragrances, topical ointments and personal care products to enhance the products "natural" appeal, and these cosmeceuticals have developed into a burgeoning market. While these topical applications do not typically produce any serious side effects, there is a potential for hypersensitivity reactions and other adverse events, such as percutaneous absorption, skin irritation, photo-toxicity or skin sensitization. The current recommended method to measure these effects is the Direct Peptide Reactivity Assay (DPRA, OECD 442C), which is itself an alternative method to animal-based methods. However, this method is relatively low-throughput, expensive and cumbersome. The DPRA method is inflexible, and limited in its ability to assess the sensitization potential of complex mixtures, such as botanical

ingredients in cosmetics. Working closely with Dr. Sadrieh and her research team at OCAC/CFSAN, NCNPR recently developed two *in-chemico* (non-biological, non-animal testing) methods for identification and classification of chemical compounds found in cosmetics and personal care products that are potential skin sensitizers. One method utilizes Nuclear Magnetic Resonance spectroscopy (NMR-method)⁹ to identify potential sensitizing compounds with their mechanistic domains. The other method uses a spectrophotometer amenable to multi-well, high-throughput screening (HTS-method)¹⁰ based on the fluorescent trapping agent, dansyl cysteamine (DCYA). The advantages over DPRA were demonstrated by testing the sensitization potential of tea tree oil,¹¹ chamomile oil¹² and pure botanical ingredients. The two described methods can be applied independently (as a stand-alone) or in combination as they complement each other in the identification of potential skin sensitizers in pure preparations, complex natural extracts or commercial formulations. As a result, of the continuation studies of the tea tree oils and potential skin sensitizers, the endoperoxide ascaridole has been identified as one of the major by-products of the oxidation of tea tree oils. Ascaridole has been allegedly identified as one of the constituent responsible for the observed increase in skin sensitization events upon exposure to aged TTOs. The reactivity of ascaridole and its fate upon radical activation were analyzed using *in chemico* methods (HTS-DCYA and NMR-DCYA). The data obtained suggest that radical activation of ascaridole generates potentially reactive compounds, including a small and extremely reactive 4-hydroxy-4-methylcyclohex-2-enone, among others, which can potentially accumulate in aged TTOs along with ascaridole.¹³ In addition to *in chemico* methods, the NCNPR also established the two other, validated, *in vitro* methods, namely, KeratinoSens (OECD 442B) and hCLAT (OECD 442D). Having all these alternative methods, botanical expertise and resources at the NCNPR, presents an ideal scenario for scientists to test the botanical ingredients used in cosmetics and other personal care products.

Another project undertaken for CFSAN's OCAC involves the generation of non-animal testing data on the skin sensitization potential of fragrance ingredients of concern. A list of more than 80 fragrance ingredients known or suspected to cause ACD has been compiled by regulatory agencies. The list may be subject to change and expansion as new data become available. Both pure compounds and natural extracts are included in the list. The long term goal of this project is to collect adequate evidence about the compounds of concern by using a combination of alternative methods. As part of this substantial effort, a first set of fragrances of concern has been evaluated. The list includes 24 pure fragrance ingredients. The compounds have been evaluated using HTS-DCYA, DPRA, KeratinoSens and hCLAT assays. A second set of additional 24 pure compounds as well as two natural extracts (*Evernia prunastri* and *E. furfuracea*) have been identified and are currently being investigated.

Additional project was undertaken to investigate the chemical stability of the fragrance ingredients of concern. Many of these fragrance ingredients do not contain the structural requirements to act as potential allergen and hence they are classified as pre- or pro-haptens. Chemical or biological activation may be involved in the generation of the "true" allergen(s). In order to establish whether chemical instability may be related to the reported ACD adverse effects, the 24 fragrance ingredients (set # 1) have been subjected to forced degradation studies by a combination of light and air exposure. The chemical stability of the fragrance ingredients has been tested using GC-MS and the sensitization potential was evaluated with both HTS-DCYA and DPRA. Several fragrance ingredients were found to be unstable; resulting in enhanced reactivity in both HTS-DCYA and DPRA assays and the complete results will be published shortly.

Another project was undertaken to investigate applicability of alternative methods in estimating the skin sensitization potential of complex mixtures, such botanical extracts. At present, a priority list of 10 plants of potential concern (based on human data, plants containing known allergens, relevance to natural hair dyes, etc.) for skin sensitization has been identified, acquired and extraction experiments are ongoing. After extraction, the whole mixture will be subjected to *in chemico* and *in vitro* alternative methods. Further analytical investigations and isolation may be required for authentication of the botanical material and identification of the constituent(s) suspected to cause ACD. The combination of innovative in-house methods along with validated methods may serve as a proactive, orthogonal testing strategy for OCAC/CFSAN's mission to address the safety assurance of botanicals in cosmetics without animal testing.

Pharmacokinetics and Herb-Drug Interactions: As a part of phytochemical investigations, scientists at NCNPR have isolated several known and previously unknown alkaloids from *Sceletium tortuosum*. Significantly, these marker components will assist the Center in authentication, identification and development of analytical

methods for *S. tortuosum* and its principal alkaloid components. *S. tortuosum*, is an indigenous herb of South Africa which is widely used as an herbal supplement in the treatment of anxiety and stress. Mesembrenone and mesembrine are the two main pharmacologically active alkaloids present in the extract, and a sensitive analytical method for simultaneous quantification of mesembrenone and mesembrine in mouse plasma was developed. The method was validated and successfully applied to evaluate the IV plasma pharmacokinetics of mesembrine and mesembrenone in mice.¹⁴

Kratom (*Mitragyna speciosa*), a native herb of Southeast Asia, is widely known for its psychoactive properties. Recent increase in the use of kratom as a recreational drug has enhanced the risk of its interaction with conventional drugs if taken concomitantly. A few reports are available related to the effects of kratom on the activity of cytochrome P450 enzymes (CYPs) but there are no reports of its effects on Pregnane X Receptor (PXR), a transcription factor that regulates the expression of CYPs and P-glycoprotein (P-gp). This study was carried out to evaluate the effects of a methanolic extract of Kratom leaves, an alkaloid-rich fraction and its five indole and four oxindole alkaloids on PXR activation and the resulting changes in the mRNA expression of PXR target genes (CYP3A4, CYP1A2, and P-gp). A significant activation of PXR was observed by the extract (3 fold), alkaloidal fraction (4 fold) and all nine alkaloids (4-6 fold) which is associated with an increased mRNA expression that resulted in an increase in the activity of CYP3A4, CYP1A2 and P-gp. These results indicate that high consumption of *Mitragyna speciosa* extract may lead to potential drug interactions due to its effects on PXR.

Aim 5: Coordinate scientific workshops and conferences on botanical ingredient-related topics of public health relevance to address high priority science and public awareness of emerging problems associated with botanicals.

Since the inception of this cooperative research program, the NCNPR has hosted seventeen scientific workshops/conferences to bring together members of industry, academia and governmental organizations. These meetings have provided a much-needed mechanism for extensive interactions between these disparate fields involving botanical supplements. Attendance of these conferences started with around 40 attendees; however, since its inception it has gradually expanded in scope, popularity and interest. Over the past few years the abstracts for this conference have been published in *Planta Medica* highlighting the importance of this conference and the impact it has had on the overall botanical scientific community.

The Center hosted the 17th Oxford International Conference on the Science of Botanicals (ICSB) that was held on April 3rd – 6th, 2017 at The University of Mississippi. The main theme of the conference was progress in botanical research and development, as well as regulatory and clinical aspects. This year's annual meeting commenced with our Program Officer, Dr. Welch's keynote address on "The Importance of Science to a Regulatory Agency". Dedicated sessions on 'Update and Future Perspectives from the FDA'; 'Natural Product Discovery and Regulation'; 'GMP: Industry Perspectives'; 'Clinical Toxicology Investigations Impacting Supplement Safety Surveillance'; 'Natural Products and Cosmetics'; 'Future Initiatives on Dietary Supplements' and 'Prospects for Naturally Derived Cannabinoids as FDA Regulated Therapeutics' are some of the highlighted sessions conducted in this year's annual meeting. All the conference proceedings including oral and poster abstracts for the 17th ICSB were posted at our website, <http://www.oxfordicsb.org/index.php>. This conference was co-sponsored by the Shanghai Institute of Materia Medica/CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant and Natural Products Research (GA); the Korean Society of Pharmacognosy and the Vietnam Academy of Science and Technology (VAST) the St Petersburg Institute of Pharmacy; the Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD) and the International Council on Medicinal and Aromatic Plants for Human Welfare (ICMAP) included representative delegations of scientists from various organizations in China, India and Europe.

Lastly, it is important to note that none of this research could have been completed without extensive leveraging of resources (financial, institutional and international). Over the past few years, the NCNPR has cultivated a significant number of collaborative interactions both nationally and internationally. The highlight of these interactions includes the formation of the Sino-US TCM Research Center Shanghai Institute of Materia Medica within the Chinese Academy of Science (SIMM/CAS); Indo-US Center for the Research of Indian Systems of Medicine (CRISM) with the Indian Council of Scientific & Industrial Research (CSIR) and with the Empresa Brasileira de Pesquisa Agropecua, Brazil. Additionally, the NCNPR has been able to expand its

research base into other related research areas by collaborating on grants with other institutes to study botanical/medicinal plants of interest. The research facilities at the NCNPR has been extended further by expanding the medicinal plant garden as well as Phase II of the NCNPR facilities with additional 110,000 square feet of research area to the existing Phase I facility. Both of these expansions will provide additional capabilities and resources to the FDA's NCNPR-COE. The creation of the Natural Products Training Center (NPTC) is a direct leverage of FDA's cooperative agreement. The NPTC has been developed through collaboration between the University of Mississippi and Waters Corporation wherein a prestigious, domestic commercial vendor furnished modern analytical instrumentation and the physical lab space was provided by the state of Mississippi. Similarly, another prominent analytical instrumentation vendor, Agilent Technologies, provided modern, state-of-the-art instrumentation for research needs of NCNPR on a collaborative basis. As a result of the close collaboration between these and other analytical instrument vendors, the instrumentation available at this FDA's COE is exceptional. These invaluable, vendor-supported efforts allowed us to provide hands-on training in analytical techniques and quality standards for botanical experts in academia, government and industry to acquire meaningful experience in addressing the issues with quality and safety of botanicals.

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C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
N/A: Not NIH Funded	Avonto C, Chittiboyina AG, Wang M, Vasquez Y, Rua D, Khan IA. In Chemico Evaluation of Tea Tree Essential Oils as Skin Sensitizers: Impact of the Chemical Composition on Aging and Generation of Reactive Species. Chemical research in toxicology. 2016 July 18;29(7):1108-17. PubMed PMID: 27286037.
N/A: Not NIH Funded	Avonto C, Chittiboyina AG, Rua D, Khan IA. A fluorescence high throughput screening method for the detection of reactive electrophiles as potential skin sensitizers. Toxicology and applied pharmacology. 2015 December 1;289(2):177-84. PubMed PMID: 26455772.
N/A: Not NIH Funded	Avula B, Chittiboyina AG, Wang YH, Sagi S, Raman V, Wang M, Khan IA. Simultaneous Determination of Aegeline and Six Coumarins from Different Parts of the Plant Aegle marmelos Using UHPLC-PDA-MS and Chiral Separation of Aegeline Enantiomers Using HPLC-ToF-MS. Planta medica. 2016 April;82(6):580-8. PubMed PMID: 27054911.
N/A: Not NIH Funded	Chittiboyina AG, Avonto C, Khan IA. What Happens after Activation of Ascaridole? Reactive Compounds and Their Implications for Skin Sensitization. Chemical research in toxicology. 2016 September 19;29(9):1488-92. PubMed PMID: 27513446.
N/A: Not NIH Funded	Chittiboyina AG, Avonto C, Rua D, Khan IA. Alternative testing methods for skin sensitization: NMR spectroscopy for probing the reactivity and classification of potential skin sensitizers. Chemical research in toxicology. 2015 September 21;28(9):1704-14. PubMed PMID: 26225548.
N/A: Not NIH Funded	Manda VK, Avula B, Chittiboyina AG, Khan IA, Walker LA, Khan SI. Inhibition of CYP3A4 and CYP1A2 by Aegle marmelos and its constituents. Xenobiotica; the fate of foreign compounds in biological systems. 2016;46(2):117-25. PubMed PMID: 26247834.
N/A: Not NIH Funded	Manda VK, Avula B, Ashfaq MK, Abe N, Khan IA, Khan SI. Quantification of mesembrine and mesembrenone in mouse plasma using UHPLC-QToF-MS: Application to a pharmacokinetic study. Biomedical chromatography : BMC. 2017 March;31(3). PubMed PMID: 27526669.
N/A: Not NIH Funded	Wang M, Carrell EJ, Chittiboyina AG, Avula B, Wang YH, Zhao J, Parcher JF, Khan IA. Concurrent supercritical fluid chromatographic analysis of terpene lactones and ginkgolide acids in Ginkgo biloba extracts and dietary supplements. Analytical and bioanalytical chemistry. 2016 July;408(17):4649-60. PubMed PMID: 27129974.
N/A: Not NIH Funded	Zhang Z, Ali Z, Khan SI, Khan IA. Cytotoxic monacolins from red yeast rice, a Chinese medicine and food. Food chemistry. 2016 July 1;202:262-8. PubMed PMID: 26920293.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
IKHLAS	Y	Khan, Ikhlas Ahmad	PHD	PD/PI	5	0	0			NA
CHITTIBOYINA	Y	Chittiboyina, Amar G.	MS,PHD	PD/PI	7	0	0			NA
	N	Hopper, Steven		Web Developer	12	0	0			NA
	N	Jayaratna, Lal		R&D Botanist	12	0	0			NA
	N	Taylor, Jennifer		Program Coordinator	12	0	0			NA
	N	Ali, Zulfiqar	PhD	Staff scientist (Doctoral level)	11	0	0			NA
	Y	Khan, Shabana	PhD	Co-Investigator	3	0	0			NA
	N	Osman, Ahmad	PhD	Staff scientist (Doctoral level)	12	0	0			NA
	N	Raman, Vijayasankar	PhD	Staff scientist (Doctoral level)	12	0	0			NA
	N	Techen, Natascha	PhD	Staff scientist (Doctoral level)	11	0	0			NA
	N	Wang, Mei	PhD	Staff scientist (Doctoral level)	6	0	0			NA
	N	Wang, Yan-Hong	PhD	Staff scientist (Doctoral level)	10	0	0			NA
	N	Zhao, Jianping	PhD	Staff scientist (Doctoral level)	8	0	0			NA
BAVULA	Y	Avula, Bharathi	PhD	Co-Investigator	4	0	0			NA
CAVONTO	N	Avonto, Cristina	PhD	Staff scientist (Doctoral level)	12	0	0			NA
IPARVEEN	N	Parveen, Iffat	BS,MS,OT H,PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	12	0	0			NA
JIYOUNG7	N	Bae, Ji-Yeong	PhD	Postdoctoral Scholar, Fellow, or Other	6	0	0			NA

				Postdoctoral Position						
MHHARON	N	Haron, Mona H.	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	12	0	0			NA
SAQLAINCHEM 2006	N	Haider, Saqlain	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	12	0	0			NA
VKUMAR2017	N	Kumar, Vikas	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	12	0	0			NA

Glossary of acronyms:

S/K - Senior/Key
 DOB - Date of Birth
 Cal - Person Months (Calendar)
 Aca - Person Months (Academic)
 Sum - Person Months (Summer)

Foreign Org - Foreign Organization Affiliation
 SS - Supplement Support
 RE - Reentry Supplement
 DI - Diversity Supplement
 OT - Other
 NA - Not Applicable

D.2 PERSONNEL UPDATES**D.2.a Level of Effort**

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

No

D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

E. IMPACT**E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?**

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS**F.3.a Human Subjects**

File uploaded: IKhan FDA change in vert animals 063017.pdf

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

F.3.b. Change in Vertebrate Animals

The original Research Strategy included proposed work with vertebrate animals. However, for the upcoming year, we do not anticipate vertebrate animal work being conducted as part of this project. A need for vertebrate animal studies in the coming year may arise with discussion with FDA project officer. In the event vertebrate animal studies are conducted in the coming year, an existing IACUC protocol is already in place

G. SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS**G.4.a Does the project involve human subjects?**

No

G.4.b Inclusion Enrollment Data

NOTHING TO REPORT

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

No

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: The University of Mississippi	067713560	MS-001	Thad Cochran Research Center and Faser Hall University MS 386771848
Missouri Botanical Garden	075914887	MO-001	4344 Shaw Blvd St. Louis MO 631102226
THE UNIVERSITY OF MISSISSIPPI	067713560		UNIVERSITY OF MISSISSIPPI 100 BARR HALL UNIVERSITY MS 386770907
The University of	067713560	MS-001	Thad Cochran Research Center and Faser Hall

Mississippi			University MS 386771848
Missouri Botanical Garden	075914887	MO-001	4344 Shaw Blvd St. Louis MO 631102226

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period?

No

G.12 F&A COSTS

Not Applicable

RPPR

RESEARCH & RELATED BUDGET - SECTION A & B

FINAL

ORGANIZATIONAL DUNS*: 067713560

Budget Type*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF MISSISSIPPI

Start Date*: 09-01-2017

End Date*: 08-31-2018

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary (\$)	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits (\$)*	Funds Requested (\$)*
1. Dr	Ikhlas	A	Khan		Project Lead	(b) (6)	4.8					
2. Dr	Amar	G	Chittiboyina		Co Project Lea	(b) (6)	6.0					
3. Dr	Shabana		Khan		Staff scientist (Doctoral level)	(b) (6)	3.0					
4. Dr	Bharathi		Avula		Staff scientist (Doctoral level)	(b) (6)	3.6					
5. Dr	Yan-Hong		Wang		Staff scientist (Doctoral level)	(b) (6)	6.6					
6. Dr	Natascha		Techen		Staff scientist (Doctoral level)	(b) (6)	9.0					
7. Dr	Zulfiqar		Ali		Staff scientist (Doctoral level)	(b) (6)	9.0					
8. Dr	Ahmad		Osman		Staff scientist (Doctoral level)	(b) (6)	9.6					
9. Dr	Jianping		Zhao		Staff scientist (Doctoral level)	(b) (6)	7.2					
10. Dr	Vijayasankar		Raman		Staff scientist (Doctoral level)	(b) (6)	10.2					
11. Dr	Mei		Wang		Staff scientist (Doctoral level)	(b) (6)	5.4					
12. Dr	Cristina		Avonto		Staff scientist (Doctoral level)	(b) (6)	9.0					
Total Funds Requested for all Senior Key Persons in the attached file												

Additional Senior Key Persons:

File Name:

Total Senior/Key Person

623,187.00

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
4	Post Doctoral Associates	26.1					(b) (6)
2	Graduate Students	24.0					(b) (6)
2	Undergraduate Students	8.0					(b) (6)
	Secretarial/Clerical						(b) (6)

RPPR	R&D Botanist Web Developer Program Coordinator	31.8	(b) (6)	FINAL	(b) (6)	
11	Total Number Other Personnel			Total Other Personnel		352,720.00
				Total Salary, Wages and Fringe Benefits (A+B)		975,907.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 067713560

Budget Type*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF MISSISSIPPI

Start Date*: 09-01-2017

End Date*: 08-31-2018

C. Equipment Description

List items and dollar amount for each item exceeding \$5,000

Equipment Item	Funds Requested (\$)*
1. GC-QToF Equipment	158,190.00
Total funds requested for all equipment listed in the attached file	0.00
Total Equipment	158,190.00

Additional Equipment: File Name:

D. Travel**Funds Requested (\$)***

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	28,000.00
2. Foreign Travel Costs	20,000.00
Total Travel Cost	48,000.00

E. Participant/Trainee Support Costs**Funds Requested (\$)***

1. Tuition/Fees/Health Insurance	0.00
2. Stipends	0.00
3. Travel	0.00
4. Subsistence	0.00
5. Other:	
0 Number of Participants/Trainees	Total Participant Trainee Support Costs
	0.00

RESEARCH & RELATED Budget (C-E) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 067713560

Budget Type*: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF MISSISSIPPI

Start Date*: 09-01-2017

End Date*: 08-31-2018

F. Other Direct Costs		Funds Requested (\$)*
1. Materials and Supplies		171,817.00
2. Publication Costs		0.00
3. Consultant Services		0.00
4. ADP/Computer Services		0.00
5. Subawards/Consortium/Contractual Costs		59,987.00
6. Equipment or Facility Rental/User Fees		0.00
7. Alterations and Renovations		0.00
8. Contractual Services		140,000.00
9. Graduate Tuition		16,380.00
Total Other Direct Costs		388,184.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	1,570,281.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1. F&A -MTDC	44.0	1,335,725.00	587,719.00
Total Indirect Costs			587,719.00
Cognizant Federal Agency			
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	2,158,000.00

J. Fee	Funds Requested (\$)*
	0.00

K. Budget Justification*	File Name:
	Budget_Justification-2017-2018_v4.pdf
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

A. PERSONNEL: \$975,907.00**Faculty and Professional Staff**

PI, Dr. Ikhlas A. Khan, Director and Research Professor, National Center for Natural Products Research, Principal Investigator will devote 40% (4.8 calendar months) of his time to this co-operative agreement. Dr. Khan will be the contact point with NCNPR for interactions with FDA scientists. Dr. Khan has extensive research project administrative experience, has served as PI of several projects. Dr. Khan is currently working as a program director of FDA program at the Center. Dr. Khan will organize and coordinate the Steering Committee operation, and work closely with FDA officials and University administration to ensure administrative compliance and performance.

Co-PI, Dr. Amar G. Chittiboyina, Assistant Director, NCNPR – 50% (6.0 calendar months) effort. Dr. Chittiboyina will be responsible for all aspects of data management for the project. Dr. Chittiboyina will coordinate particularly with the botanists, geneticist, isolation, analytical chemistry investigators and biologists, as well as with FDA scientists involved in the project, to develop and modify the data management workflow. Additionally Dr. Chittiboyina is on the organizing committee for the Annual Oxford International Conference on the Science of Botanicals (www.oxfordICSB.org). He works directly with Dr. Khan on a daily basis for scientific direction of major portions of NCNPR research efforts

Principal Scientist – (Dr. Shabana I. Khan) - 25% (3.0 calendar months) effort. Dr. Khan will be responsible for direction of the efforts at UM to evaluate the ADME, herb-drug, toxicological parameters for the natural products and botanical extracts. Dr. Khan's training is in biochemistry, and she has worked for many years in the screening development with natural products.

Senior Research Scientist, Analytical Chemist – (Dr. Bharathi Avula) – 30% (3.6 calendar months) effort. Research Scientist, National Center for Natural Products Research will be responsible for analytical method development. Dr. Avula is a trained pharmacist and analytical chemist. She has developed expertise over the years in analysis of botanicals. She will also be supervising Dr.'s Yan Hong Wang and Mei Wang in developing relevant analytical methods.

Senior Research Scientist, Analytical Chemist – (Dr. Yan Hong Wang) – 55% (6.6 calendar months) effort. Dr. Wang will be responsible for analytical method development. Dr. Wang is a natural products chemist with analytical background. He has developed an extensive expertise over the years in the analysis of botanicals.

Research Scientist, Plant Genetics – (Dr. Natascha Techen) – 75% (9.0 calendar months) effort. Dr. Techen will be responsible for plant genetics approaches applied to the authentication of botanical dietary supplements. She is experienced in all the molecular techniques needed to accomplish this task.

Research Scientist, Isolation Chemist – (Dr. Zulfiqar Ali) – 75% (9.0 calendar months) effort. Dr. Ali will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Sr. Research Scientist, Chemist – (Ahmad Osman) 80% (9.6 calendar months) effort. This individual will be responsible for isolating and identifying compounds of interest from various botanical sources. Additionally, this person will be instrumental in compiling scientific information with regard to identification techniques (analytical, NMR, etc.) for the authentication of botanical dietary supplements for the purpose of populating a dynamic web portal to assist FDA researchers and other stakeholders.

Research Scientist, Isolation Chemist – (Dr. Jianping Zhao) – 60% (7.2 calendar months) effort. Dr. Zhao's responsibility will be to prepare extracts of selected botanicals, develop NMR identification/authentication techniques, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards. Dr. Zhao has several years of experience in developing analytical HPTLC techniques for the purpose of analysis and quantitation of various botanical ingredients in dietary supplements.

Research Scientist, Botanist – (Dr. Vijayasankar Raman) – 85% (10.2 calendar months) effort. Dr. Raman is responsible for living collection in medicinal plant garden and he is also an expert in taxonomy. He has setup a network of botanical institutes to coordinate collection effort and germplasm/ seed bank collections which is required for collection, identification and authentication of medicinal plants of interest, including wild collections and cultivated specimens. Dr. Raman will be working on macro and microscopy of botanicals, needed for species authentication. Microscopy provides an understanding of anatomical features of a plant, which is instrumental in differentiating the species and also in determining potential adulterants.

Research Scientist, Analytical Chemist – (Dr. Mei Wang) – 45% (5.4 calendar months) effort. Dr. Wang will develop analytical methods for the purpose of identifying botanical dietary supplement samples. Dr Wang has several years of experience in developing analytical GC techniques for the purpose of analysis and quantitation.

Research Scientist, Chemist – (Dr. Cristina Avonto) – 75% (9.0 calendar months) effort. Dr. Avonto will be responsible for the development of non-animal alternative methods for skin sensitization and to evaluate the sensitization potential of botanical ingredients in cosmetics and personal care products using *in chemico* and *in vitro* assays.

Post-Doctoral Research Associate, Isolation and Analytical Chemist – (Dr. Ji-Yeong Bae) – 75% (4.5 calendar months) effort. Dr. Bae will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards. Assist Dr. Avula on method development and method validation for botanicals.

Post-Doctoral Research Associate, Plant Genetics – (Dr. Iffat Parveen) – 75% (9.0 calendar months) effort. Dr. Parveen will be responsible for plant genetics approaches applied to the authentication of botanical dietary supplements. She will assist and train under Dr. Tehen for the molecular techniques needed to accomplish the proposed work.

Post-Doctoral Research Associate, Synthetic Chemist – (Dr. Saqlain Haider) – 45% (5.4 calendar months) effort. Dr. Haider will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards. In addition to isolation work, he will be responsible for identification and synthesis of small molecules hepatotoxicity.

Post-Doctoral Research Associate, Biology – (Dr. Vikas Kumar) – 60% (7.2 calendar months) effort. Dr. Kumar will be responsible for the development of *in-vitro* assays to assess the safety of botanical ingredients used in supplements, cosmetics and other personal care products.

R&D Botanist – (Lal Jayaratna) – 85% (10.2 calendar months) effort. This individual is responsible for maintaining the living collection of the centers medicinal plant garden. Additionally this individual prepares voucher specimens, maintains the programs seed bank and provides assistance/tours for training sessions and workshops.

Web Developer – (Steven Hopper) 90% (10.8 calendar months) effort. This individual will be responsible for the development of a botanical dietary supplement information portal for the dissemination of relevant scientific data and FDA Dietary Supplement cGMP information. In addition to this he will be aiding in the data labeling, collection/reporting efforts for this project.

Technical and Administrative Staff

Program Coordinator (Jennifer Taylor) - 90% (10.8 calendar months) effort. Ms. Taylor is responsible to the PIs, to allow for adequate follow-up with reports, publications, file documentation, sample tracking, etc. Ms. Taylor also instrumental in vital logistical support for workshops, training sessions and conferences.

NOTE: The position of Program Coordinator is normally not allowed as direct costs under OMB circular A-21. However, we are requesting these direct costs be allowed due to the large scope of the project and the number

of personnel to be managed and supported. This position is easily allocable to the project, and is reasonable given the size and nature of the project.

Hourly Wages – Hourly wage support will be utilized for temporary and student workers employed in support of the project. These may be utilized in support of administrative or research activities, ranging from filing and data entry to field/greenhouse or laboratory assistance.

Graduate Students (2) – The graduate students will receive training in natural products chemistry, analytical chemistry. These students will be enrolled in the Ph.D. program in the Department of Pharmacognosy, or one of the other academic Departments in the school of Pharmacy.

Fringe Benefits

Fringe benefits for faculty and staff are calculated at the University's average rate of 32.80% of salary. Fringe benefits for graduate research assistants are calculated at the University's average rate of 8.0% of stipend. Fringe benefits for students paid hourly (graduate or undergraduate) are calculated at the University's average rate of 3.0% of wages. The University uses average rates for budgeting fringe benefits; however, charges made to the sponsor will be for actual fringe benefits paid per individual.

B. CONSULTANT COSTS: None

C. EQUIPMENT: \$158,190

These funds are to support the capacity for analytical work that is required for the success of the program. These funds will be utilized to either purchase new complete systems or to upgrade other existing equipment HPLC, GC, CE or MS.

D. SUPPLIES: \$ 171,817

Primary commodity expenditures for the project will be for:

HPLC columns \$20,000

NMR/MS supplies (tubes, gases, columns) \$11,000

Chemicals, Reagents and Standards \$15,000

Microscopic supplies (slides, stains, storage cassettes) \$10,000

Chromatographic supplies (TLC plates, chromatographic media, solvents) \$49,817

Mol. Biology supplies \$20,000

Botanical collection/storage materials \$15,000

Garden/greenhouse tools/supplies \$ 15,000

Books, databases other reference materials \$8,000

Computer supplies \$8,000**

****These costs are for essential computer supplies which are devoted solely to the FDA project, and not for general use.

E. TRAVEL: \$48,000

Included in this category are:

Travel for FDA Dietary Supplement GMP training sessions.

Establishing collection arrangements and collaborations (National and International)

Travel to/from Washington or other COE's for meetings with FDA officials

Travel costs related to hosting workshop/conference (National and International)

F. CONTRACTUAL SERVICES: \$140,000

Estimated expenditures in this category include contractual/professional services for:

Collection & documentation of plant material \$8,000

Scale-up extraction/isolation \$ 9,000

Taxonomic verifications \$8,000

Maintenance contracts/repairs for analytical equipment \$45,000

Software/upgrades for analytical equip. \$12,000

Shipping, mailing costs \$4,000

Sub-Total: \$86,000

Estimated expenses for hosting conference:

Printing/PR \$8,000

Speaker reimbursements (20 @ 1,500) \$30,000

Dinners/breaks \$ 10,000

Staffing \$ 6,000

Sub Total: \$54,000

G. SUBCONTRACT: \$ 59,987

A subcontract with Missouri Botanical Garden will be in place for the amount of \$ 59,987. Missouri Botanical Garden will be collecting the specimens for this project and will be providing expertise on identification of the specimens. Dr. Rainer Bussmann, the director and curator of the William L. Brown Center for Plant Genetic Resource (WLBC), will be the Missouri Botanical Garden representative working on the project.

H. INDIRECT CHARGES: \$ 587,719

Indirect costs are calculated in accordance with The University of Mississippi's rate agreement with DHHS, dated September 12, 2011. Indirect costs for research are calculated at 44.0% of Total Direct Costs less equipment, tuition remission, and the portion of each sub-grant or subcontract in excess of \$25,000.

RPPR

RESEARCH & RELATED BUDGET - SECTION A & B

FINAL

ORGANIZATIONAL DUNS*: 075914887

Budget Type*: ☐ Project ☒ Subaward/Consortium

Enter name of Organization: Missouri Botanical Garden

Start Date*: 09-01-2017 End Date*: 08-31-2018

A. Senior/Key Person													
Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary (\$)	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits (\$)*	Funds Requested (\$)*	
1. Dr	Rainer		Bussmann		Consortium PI	0.00	0.01			0.00	0.00	0.00	
Total Funds Requested for all Senior Key Persons in the attached file													
Additional Senior Key Persons:			File Name:								Total Senior/Key Person		0.00

B. Other Personnel							
Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary (\$)*	Fringe Benefits*	Funds Requested (\$)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	Research Specialists	9.0			24,000.00	5,560.00	29,560.00
3	Total Number Other Personnel					Total Other Personnel	29,560.00
					Total Salary, Wages and Fringe Benefits (A+B)		29,560.00

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 075914887

Budget Type*: ☐ Project ☒ Subaward/Consortium

Enter name of Organization: Missouri Botanical Garden

Start Date*: 09-01-2017

End Date*: 08-31-2018

C. Equipment Description

List items and dollar amount for each item exceeding \$5,000

Equipment Item	Funds Requested (\$)*
Total funds requested for all equipment listed in the attached file	0.00
Total Equipment	0.00
Additional Equipment: File Name:	

D. Travel

Funds Requested (\$)*

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,500.00
2. Foreign Travel Costs	17,000.00
Total Travel Cost	19,500.00

E. Participant/Trainee Support Costs

Funds Requested (\$)*

1. Tuition/Fees/Health Insurance	0.00
2. Stipends	0.00
3. Travel	0.00
4. Subsistence	0.00
5. Other:	
0 Number of Participants/Trainees	Total Participant Trainee Support Costs
	0.00

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 075914887

Budget Type*: ☐ Project ☒ Subaward/Consortium

Enter name of Organization: Missouri Botanical Garden

Start Date*: 09-01-2017

End Date*: 08-31-2018

F. Other Direct Costs		Funds Requested (\$)*
1. Materials and Supplies		3,500.00
2. Publication Costs		0.00
3. Consultant Services		0.00
4. ADP/Computer Services		0.00
5. Subawards/Consortium/Contractual Costs		0.00
6. Equipment or Facility Rental/User Fees		0.00
7. Alterations and Renovations		0.00
8. Contractual-Specimen shipping		1,000.00
Total Other Direct Costs		4,500.00

G. Direct Costs	Funds Requested (\$)*
Total Direct Costs (A thru F)	53,560.00

H. Indirect Costs			
Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base (\$)	Funds Requested (\$)*
1. Modified Total Direct Costs	12.0	53,561.00	6,427.00
Total Indirect Costs			6,427.00
Cognizant Federal Agency			
(Agency Name, POC Name, and POC Phone Number)			

I. Total Direct and Indirect Costs	Funds Requested (\$)*
Total Direct and Indirect Institutional Costs (G + H)	59,987.00

J. Fee	Funds Requested (\$)*
	0.00

K. Budget Justification*	File Name: MOBOT_Budget Justification FDA 17-18_v2.pdf (Only attach one file.)
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RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification

Salaries

Salary is requested for three months offsite each for three field botanists, at Senior Herbarium Assistant rank, who will assist Dr. Bussmann with collection, identification, and processing of all plant specimens and samples.

A fringe benefit rate of 23.17% is applied to each of the salaries. The Garden's fringe benefit package includes payroll taxes, health insurance, dental insurance, life insurance, TDA, and long-term disability insurance.

Supplies

For field collection an amount of \$ 3,500 is requested for this year (September 2017- August 2018). The project requires supply needs and \$ 1,000/year is requested for specimen shipping costs.

Travel

Support is requested for two botanists to conduct four one-week field trips within the United States, and one four-week field trip each to Peru, Bolivia and the Caucasus per year. Costs are calculated on the basis of an average cost to \$275 per day for the team of two overseas.

Indirect Costs

The Missouri Botanical Garden uses indirect cost rates approved as appropriate by the National Science Foundation, which are 60% on-site and 12% off-site.

Progress Report Summary

A. Specific Aims

Under the provisions of the DSHEA, the FDA has primary responsibility for ensuring that appropriate regulatory actions are taken against marketed products that present significant health risks or bear false or misleading label claims. Foundational to the evaluation of such risks and claims is an adequate scientific base for decision-making. For botanical dietary supplements, development of such a science base is especially problematic because of several unique features, including the complexity of the constituents, variability of sourcing, lack of availability of reference materials, lack of manufacturing controls and rapidly expanding use in the marketplace. In 2010 the FDA fully implemented the current good manufacturing practices (cGMP) for the botanical dietary supplement industry placing the onus of “botanical identity and authenticity” on the manufacturers of botanical dietary supplements. However, these cGMP’s have in many ways increased the complexity as to what constitutes a “scientifically valid method” for the determination of the identity and authenticity of botanical materials. To address these complex issues surrounding botanical identity, authenticity, and safety and in continuation of our cooperative agreement with the FDA, the NCNPR has established the following specific aims to aid in the research needs of the FDA:

1. Assist in the identification and development of a list of BDS and botanical ingredients based on safety concerns, trends, and knowledge of botanicals being marketed in the U.S., to prioritize for further research.
2. Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to FDA, for evaluation of their safety.
3. Exchange technical and scientific information, analytical methods, and reference material with FDA scientists and other stakeholders.
4. Collaborate with FDA scientists in research areas of mutual interest.
5. Coordinate scientific workshops and conferences on BDS-related topics of public health relevance to address high priority science and research needs.

This renewal request expands the research under the current agreement on BDS to include potential safety issues, and extends the effort to include additional emerging problems associated with botanicals.

B. Studies and Results:

Aim 1: Assist in the identification and development of a list of BDS and botanical ingredients based on safety concerns, trends, and knowledge of botanicals being marketed in the U.S., to prioritize for further research.

Since the inception of the cooperative agreement between the NCNPR and the FDA in 2001, there have been numerous occasions for interactions between the two organizations. These have included (yearly) formal site visits by the FDA program officers, interactions during the annual International Conference on the Science of Botanicals (ICSB) and several conference calls and email exchanges. In addition to these interactions, over the past year the NCNPR has hosted five training sessions with the Office of Regulatory Affairs (ORA) to provide guidance to FDA inspectors for cGMP compliance issues for BDS’s (FD340). These training sessions have provided an opportunity for the programs project officer (Dr. Daniel Fabricant) to visit the NCNPR and stay abreast of the Centers ongoing developments. As always, researchers at the NCNPR are receptive to areas of study that are deemed necessary by the FDA for this project.

Aim 2: Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to FDA, for evaluation of their safety.

The availability of authenticated reference materials for botanicals is an essential first step in the development of analytical methodologies for the identification and quantitative analysis of botanical product(s). The NCNPR has been very successful in placing formal agreements with several international academic and governmental institutions including Pakistan, India (FRLHT), South Africa, Central/South America, China with the development of the Sino-US TCM Research Center and the established Center for Research in Indian Systems of Medicine (CRISM - www.CRISM.net) with the departments of AYUSH and CSIR in India. The

NCNPR has also established a Memorandum of Understanding (MOU) with the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Brazil and the Natural Products Utilization Research Unit (NPURU), USDA located in Oxford Mississippi. Lastly the NCNPR has cultivated a relationship with the Chinese FDA in order to obtain relevant authenticated Traditional Chinese Medicines (TCM's) and pertinent purified constituents for authentication purposes yielding approximately 50 new constituents over the past year.

From these and other collaborative relationships, the NCNPR has been able to acquire over 9500 plant samples and herbal extracts, representing approximately 4500 species over the duration of this project. There is a continuous effort in acquiring commercial samples as well for authentication purposes in addition to assuring that the developed identity testing techniques for this project are applicable for finished products in various formularies. Taking all these sample types into account there are over 15,000 samples within the NCNPR-FDA repository. All the plants and samples collected for this program have been assigned an inventory number and are cataloged in the NCNPR repository and voucher specimens of authentic samples are also maintained at the Thomas M. Pullen Herbarium in the Department of Biology at the University of Mississippi (MISSA).

In addition to this the NCNPR has a newly renovated Medicinal Plant Garden that maintains more than 270 species for selected growing (field, greenhouse and shade houses). The new facilities consist of two main buildings (4,362 sf. and 4,290 sf.) and four additional support buildings and structures sitting on approximately 5.25 acres of land. The Medicinal Plant Garden has developed significant collaborations with seed banks from 40 institutes from around the world in order to exchange medicinally important germplasms. This seed bank effort has yielded a collection of over 800 species to date. In addition, the garden personnel are preparing herbarium voucher specimens from the living collection and have started collecting samples for a DNA tissue bank. Over the course of this program the garden provided 320 authentic reference samples from the living collection for the Centers research efforts. The Medicinal Plant Garden is also registered under the Botanic Gardens Conservation International and is a member of the American Association of Botanical Gardens and Arboreta and International Plant Exchange Network (IPEN).

Isolation of reference compounds: The isolation of standard compounds is required to develop analytical methods, quantify individual compounds in extracts and to establish analytical fingerprints in support of quality and safety studies. Scientists at the NCNPR have isolated a number of compounds from species such as *Matricaria recutita* L., *Anthemis nobilis*, *Terminalia* spp., *Dioscorea villosa*, *Dioscorea cayenensis*, and *Lepidium meyenii* (Maca). It is through these continued efforts that the NCNPR scientist have isolated dozens of novel compounds as well as known analogs for analytical finger printing development for authentication purposes as well as safety analysis.

Analytical method development and metabolomic profiling: In the past year we have focused on developing several analytical methods for the determination of authenticity BDS's of interest. These studies include the development of UPLC (UV, ELS and MS), standard HPLC/HPTLC analytical methods as well as using proton NMR for metabolomic profiling for common botanicals including Cinnamon species (*Cinnamomum verum*, *C. cassia*, *C. loureiroi*, and *C. burmannii*), *Pausinystalia johimbe*, *Pausinystalia johimbe*, *Terminalia* spp. (*T. chebula*, *T. arjuna*, *T. bellirica*), *Phyllanthus emblica*, *Dioscorea cayennensis* Lam., *Dioscorea rotundata* Poir., *Dioscorea opposita* Thunb., *Dioscorea caucasica* Lipsky, *Dioscorea villosa* L., *Dioscorea bulbifera* L., *Dioscorea deltoidea* Wall. ex Griseb., *Dioscorea quaternata*, *Lepidium meyenii* (Maca), and *Euterpe oleracea* Mart. (acai berries). Most importantly, the Center aided in developing an analytical approach establishing the absence of dimethylamylamine (DMAA) in authenticated *Pelargonium graveolens*. This newly developed method provided the FDA with the information required to challenge the marketing of DMAA products for lack of safety evidence on April 27, 2012.

Aim 3: Exchange technical and scientific information, analytical methods, and reference material with FDA scientists and other stakeholders.

Dr. Ikhlas Khan, Scientific Director for the project, has been in touch regularly with Dr. Daniel Fabricant (CFSSAN, Director, Division of Dietary Supplement Programs), Dr. Elizabeth Calvey (CFSSAN liaison), Dr. Diego

Rua (CFSAN) and Dr. Robert L. Sprando, (CFSAN/OARSA, Director, Division of Toxicology) to exchange information on developed methods, reference materials availability, safety evaluations and project direction. These meetings and calls are designed to provide these individuals with an update of the NCNPR's overall program and to gain insight as to how this Center can better address the needs of the FDA.

Additionally, the NCNPR FDA-COE has also continued to assist the FDA's Office of Regulatory Affairs (ORA) in training inspectors for cGMP compliance for BDS's (FD340). Researchers at the NCNPR provided five one-day workshops on botanical dietary supplement authentication techniques to 193 trainees and FDA officials on December 12th, 2012, March 18th, 2013, April 22nd, 2013, May 20th, 2013, and June, 19^h, 2013. The main training course is held in Memphis, Tennessee so that the trainees can attend a one-day excursion to the NCNPR for a combination of lectures and laboratory courses and training sessions to see what authentication techniques can be implemented for BDS's. The course covered current techniques utilized to identify botanical materials (Microscopy, Taxonomy, Macroscopy, TLC, HPLC, UPLC, GC, CE, etc) and was presented by Dr. Khan and colleagues at the NCNPR and included two one and one half hour lab courses on Analytical Methods, Botanical Authentication, and Nomenclature. It is anticipated that this training program will continue for the foreseeable future and will provide the FDA cGMP botanical dietary supplement inspectors a valuable tool that will assist in their ability to carry out their duties.

Researchers at the NCNPR have also provided their expertise in other training offered by the FDA/ORA/DHRD. One such course was an advanced level course for analysts who are performing regulatory sample analysis using mass spectrometry techniques for identification and authentication (LB 403). Specifically, Dr. Yan-Hong Wang provided a lecture to several FDA trainees on August 26th – 31st, 2012 covering the topic of how mass spectroscopy can be utilized for the authentication of botanicals. Dr. Wang also presented similar topics at a workshop organized by The University of Maryland and JIFSAN entitled "Dietary Supplements: Microscopic and Chemical Identification of Botanicals" on October the 15th-16th, 2012 to aid in the FDA's international food safety training program. As an extension of this joint training with JIFSAN, the NCNPR sent Dr. Suman Chandra to provide four presentations at a GAP & GMP workshop aimed at supply chain management for spices and botanical ingredients for the Indian spice board (September 17th-21st, 2012, Kochi, India). Dr. Chandra covered topics such as harvesting considerations, transportation/processing, cleaning and sanitation techniques. This workshop was then expanded into a multi-day multi-site visit for several members of the Indian spice board to provide these individuals with onsite training and lectures to provide advanced GAP and GMP techniques. This workshop, entitled "Food Safety and Supply Chain Management for Spices and Botanical Ingredients" was hosted by JIFSAN from March 25th - April 2nd then the NCNPR from April 3rd - 5th 2013.

Lastly, the research effort initiated by the establishment of this Center has provided several opportunities to leverage the existing cooperative agreement so as to expand the impact and overall benefit of the existing program. This includes a recently funded NCCAM/ODS Botanical Research Center grant to explore Botanical Estrogens: Mechanisms, Dose and Target Tissues (PD: Helferich, William G., University of Illinois/PL: Khan, Ikhlas, A., Award number 5 P50 AT006268-02). Under this grant the NCNPR is providing significant quantities and populations of authenticated samples of Licorice - *Glycyrrhiza glabra* Linné var *glabra*, Wild Yam - *Dioscorea villosa* L., and Dong Quai - *Angelica sinensis* (Oliv.) Diels for the established BRC. In addition to obtaining the outlined authenticated species for this program we have established a substantial growing and collection program for closely related species for authentication and comparative purposes. Additionally, the NCNPR has obtained funding from the USDA/ARS for a grant entitled Discovery and Development of Natural Product-Based Insect Management Compounds for Medicinal, Veterinary and Urban Concern, award number 58-6402-7-228. The purpose of this grant is to discover and develop new, safer and more effective insect management products derived from natural sources.

Aim 4: Collaborate with FDA scientists in research areas of mutual interest.

Over the past year the NCNPR has provided the FDA with scientific information for botanicals of concern as well as investigated several plants that have purported adverse events. Most notably we have undertaken an investigation of "chamomile" which has had reports of contact dermatitis and potential severe cases of allergic

reactions in cosmetic formulations. The initial question, as with all botanicals, is what is meant when discussing “chamomile” such as which species or variety was used, what plant part, what processing method was used, etc. As it turns out there are two main species of “chamomile” utilized for commerce within the United States German chamomile, *Matricaria recutita* L. and Roman chamomile, *Anthemis nobilis*. Typically the flowering tops are used for most cosmetic formulations and these are either added as powdered material or an extract (ethanolic, supercritical or steam distilled). Working closely with scientist in the FDA/CFSAN office of cosmetics and colors, we initiated an extraction and bioassay guided fractionation of both German and Roman chamomile utilizing an LLNA screening assay for lead identification. Initial results are indicated that there is a potential sensitizer within *Matricaria recutita* L. that could be causing the purported adverse events. Further investigation including isolation, purification and bioassay evaluations have been undertaken and are still in progress to identify the constituent(s) that contribute to the observed sensitization.

Additional investigations of cosmetic products that contain β -arbutin were also carried out. β -arbutin is a natural product that can be extracted from plants but can also be made synthetically. Cosmetics manufacturers are purportedly adding β -arbutin to products as a skin-whitening agent. However the EU Scientific Committee on Consumer Products (SCCP) determined in 2008 that β -arbutin in cosmetic products should be considered unsafe. In 2006, the FDA issued a notice of proposed rulemaking to establish that all skin bleaching products, whether marketed on a prescription or OTC basis, are drugs requiring an approved new drug application (NDA) for continued marketing. However the corresponding final rule has not been issued. The main safety concern for β -arbutin is that it can be a potential source for hydroquinone, which has displayed carcinogenicity in animals and poses a potential carcinogenic risk in humans. In addition, hydroquinone has been shown to cause disfiguring effects (ochronosis) after topical use at concentrations as low as 1 to 2-percent. Since β -arbutin can be extracted from plants, one general research objective was to seek further knowledge regarding which plants are best sources of β -arbutin such as *Salvia officinalis*. A developed analytical method was developed to differentiate between α -arbutin (synthetic) and β -arbutin (natural). This method was established and validated for plant sources that are known to contain arbutin, then utilized to analyze several (~30) botanical products that claimed to contain natural β -arbutin to see what levels of α and β arbutin each product contains. The results of this investigation showed that 52% and 30% products were found to contain β -arbutin and α -arbutin, respectively and that seven products did not contain β -arbutin or α -arbutin within the limits of detection. Stability testing of β -arbutin and α -arbutin indicated that while both compounds would degrade over time and under various conditions, they did not interconvert to the alternate form.

A second project undertaken for CFSAN's office of cosmetics and colors looked at products that include an essential oil known as “Tea tree oil”, which is obtained from several species of *Melaleuca* plants. One general research objective for this project is to seek further knowledge on compositional differences between commonly used species of *Melaleuca* plants (*Melaleuca alternifolia*, *Melaleuca linariifolia*, *Melaleuca viridiflora*, *Melaleuca leucadendron*, *Melaleuca dissitiflora*, etc.) in order to explore ways to address safety concerns for these species. The main safety concern about essential oils from these plant species is the potential for adverse effects on the skin, in particular sensitization or allergic contact dermatitis (ACD). It is not fully understood which of the tea tree oil constituents is responsible for ACD. Therefore, the potential ACD active(s) in tea tree oil are currently being identified utilizing an *in vitro* cell-based assay. Concurrently, we have developed an analytical GC/MS method to differentiate between the various species of tea tree oil that also identifies the major constituents. This newly developed method can be used to help identify potential ACD's within products.

Lastly, after extensive discussions with our FDA program officer, the Center established two in-house preliminary *in-vivo* safety evaluations for botanicals of interest. Priority evaluation was given to botanicals that have displayed an inherent risk based on literature information or reported adverse events. Initial screens focused on two areas of concern. The first mouse model measured botanicals for their potential to induce positive reinforcement or cause aversive properties using a conditioned place preference (CPP) paradigm procedure that is commonly used to evaluate drugs for their “addictive” behavior. Over the past year the Center used this model to evaluate *Salvia divinorum*, *Mitragyna speciosa* and fractions and pure compounds isolated from these species. For *S. divinorum* the preliminary results indicated that this particular botanical did not induce abusive potential. For *M. speciosa* the results indicated that the major pharmacologically active

constituent, mitragynine, has an abuse potential. The second *in-vivo* assay was developed to evaluate the potential hepatotoxicity's associated for botanical as measured in a mouse model where the hepatotoxicity is manifested by elevation of aminotransferases and histopathological features of acute hepatocellular necrosis and/or biliary injury. Initial *in-vivo* hepatotoxicity evaluations of EGCG, a major component in green tea products, at high doses can lead to mild liver injury and under febrile conditions it can cause severe liver injury. Both *in-vivo* models will continue to provide significant insight into the safety profile for botanicals that are of concern to public health.

Aim 5: Coordinate scientific workshops and conferences on BDS topics of public health relevance to address high priority science and research needs.

The Center hosted the 12th Oxford International Conference on the Science of Botanicals (ICSB) that was held on April 15th – 18th, 2013, at The University of Mississippi. This conference was co-sponsored by the Shanghai Institute of Materia Medica/CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant and Natural Products Research (GA); the Korean Society of Pharmacognosy and the Vietnam Academy of Science and Technology (VAST) the St Petersburg Institute of Pharmacy; the Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD) and the International Council on Medicinal and Aromatic Plants for Human Welfare (ICMAP) as such included representative delegations of scientists from various organizations in China, India and Europe. In addition to this there were representatives from several well-known international organizations as both attendees and presenters to total over 260 participants at this conference. The conference also included a specific session dedicated to highlight the scientific efforts of the established ODS/NCCAM Botanical Research Centers. The abstracts for this conference have been published in *Planta Medica*, 2013, 79(5), 369-421.

C. Significance:

Plant collection, authentication, voucher specimens, isolation of reference compounds and method development provide the basic tools for assessing the safety and quality of botanical products. Significant progress has been made by the NCNPR in each of these areas for several botanicals of interest over the past year. The acquired information is freely available to researchers at the FDA as well as physical samples (plants, extracts, etc) and phytochemical standards for evaluation purposes. Publications from this project have significantly contributed to the available methodological literature for the FDA and NCNPR (see references).

D. Plans:

Furthering the collaborative research effort we have established with CFSAN's office of cosmetics and colors, additional effort will continue to look at several potential areas of concern. The first being the continued investigation of cosmetic products that contain "arbutin"(s). The second project will entail continuing the exploration of products that contain "tea tree" essential oil(s) which can be derived from several species of *Melaleuca* plants. The main research objective for this project will be to seek further knowledge on compositional differences between commonly used species of *Melaleuca* plants (*Melaleuca alternifolia*, *Melaleuca linariifolia*, *Melaleuca viridiflora*, *Melaleuca leucadendron*, *Melaleuca dissitiflora*, etc.) in order to explore ways to address safety concerns for these species and their potential for producing ACD activity.

Continual research effort will also focus on the two recently developed in-house *in-vivo* screen evaluating botanicals for their potential to induce positive reinforcement or cause aversive properties using the developed CPP paradigm procedure that is commonly used to evaluate drugs for "addictive" behavior and the second assay which evaluates potential hepatotoxicity associated for certain botanicals. Priority evaluation will be given to botanicals that have displayed an inherent risk based on literature information or reported adverse events. Both models will provide significant insight as to the safety profile for botanicals that are of concern to the public health.

Lastly, expansion of the existing repository of reference plant samples for the genera noted throughout this report will be a continuing priority for this project. New botanicals will be selected based on input received from the FDA program officer, collaborators and liaison for further studies and to evaluate their safety and quality. A

thirteenth and fourteenth conferences are being organized. The first will be a shorter workshop entitled Oxford International Conference on the Science of Botanicals (ICSB) and will be held on April 14th – 15th, 2014 and the second will be a full conference that will be held in conjunction with the American Society of Pharmacognosy's 2014 Annual meeting on August 2nd-6th, 2014. These conferences will be co-sponsored by the Shanghai Institute of Materia Medica/CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the Ministry of Indigenous Medicine, Sri Lanka; the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant and Natural Products Research (GA); the Korean Society of Pharmacognosy and the Vietnam Academy of Science and Technology (VAST) the St Petersburg Institute of Pharmacy; the Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD) and the International Council on Medicinal and Aromatic Plants for Human Welfare (ICMAP). This conference will cover the topics of TCM, Ayurveda, cultivation, collection, post-harvest, processing practices, identification, authentication and quality/safety assessment of botanicals including current clinical evaluations, regulatory aspects and the impact on the global dietary supplement industry. Again the proceedings from these conferences are expected to be published in *Planta Medica*.

References

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Department of Health and Human Services
Public Health Services

Review Group

Type

Activity

Grant Number

1U01FD004246

Grant Progress Report

Total Project Period

From: 09/15/2011

Through: 08/31/2016

Requested Budget Period

From: 09/1/2013

Through: 08/31/2014

1. TITLE OF PROJECT

Science Based Authentication of Dietary Supplements

2a. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR

(Name and address, street, city, state, zip code)

Khan, Ikhlas, A
120 Faser Hall/NCNPR
School of Pharmacy
University of Mississippi
University, MS 38677**2b. E-MAIL ADDRESS**

ikhlan@olemiss.edu

2c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT

National Center for Natural Products Research

2d. MAJOR SUBDIVISION

School of Pharmacy

2e. Tel: 662-915-7821

Fax: 662-915-7989

3a. APPLICANT ORGANIZATION

(Name and address, street, city, state, zip code)

The University of Mississippi
Office of Research and Sponsored Programs
100 Barr Hall or PO Box 907
University MS 38677

3b. Tel: 662-915-7482

Fax: 662-915-7577

3c. DUNS: 067713560

4. ENTITY IDENTIFICATION NUMBER

1646001159A1

6. HUMAN SUBJECTS☒ No ☐ Yes6a. Research
Exempt☒ No ☐ YesIf Exempt ("Yes" in
6a):

Exemption No.

If Not Exempt ("No" in
6a):

IRB approval date

5. NAME, TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIALDr. Robin C. Buchannon, Assistant Vice Chancellor,
Office of Research and Sponsored Programs
100 Barr Hall or PO Box 907, University MS 38677

Tel: 662-915-7482

Fax: 662-915-7577

E-MAIL: research@olemiss.edu

6b. Federal Wide Assurance No.

6c. NIH-Defined Phase III

Clinical Trial ☒ No ☐ Yes**7. VERTEBRATE ANIMALS** ☐ No ☒ Yes

7a. If "Yes," IACUC approval Date 06-20-12

7b. Animal Welfare Assurance No. A3356-01

10. PROJECT/PERFORMANCE SITE(S)

Organizational Name: The University of Mississippi

DUNS: 067713560

8. COSTS REQUESTED FOR NEXT BUDGET PERIOD

8a. DIRECT \$1,590,767

8b. TOTAL \$2,495,000

Street 1: 120 Faser Hall/NCNPR

Street 2: School of Pharmacy

9. INVENTIONS AND PATENTS ☒ No ☐ YesIf "Yes," ☐ Previously Reported☐ Not Previously Reported

City: University

County: Lafayette

State: MS

Province:

Country: USA

Zip/Postal Code: 38677

Congressional Districts: MS-001

11. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Item 13)

Dr. Alice M. Clark, Vice Chancellor, Office of Research and Sponsored Programs

TEL: 662-915-7482

FAX: 662-915-7577

E-MAIL: research@olemiss.edu

12. Corrections to Page 1 Face Page

13. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN
11. (In ink)

DATE

7/3/13

Detailed Budget for Next Budget Period

			Salary Req.	Fringe	Total
Personnel					
PI	Ikhlas Khan	45%	(b)	(6)	
Co-PI	Larry Walker	10%			
Sr. Research Scientist	Shabana Khan	23%			
Sr. Research Scientist	Troy Smillie	45%			
Res. Scientist	Bharthi Avula	70%			
Sr. Research Scientist	Yan Hong Wang	100%			
Res. Scientist	Natascha Techen	100%			
Res. Scientist	Zulfiqar Ali	25%			
Res. Scientist	Amar Chittiboyina	50%			
Res. Scientist	Gouyi Ma	100%			
Res. Scientist	Ahmad Osman	100%			
Assoc. Res. Scientist	Jianping Zhou	53%			
Post Doc	Vijayasankar Raman	100%			
Post Doc	Mei Wang	70%			
Post Doc	Cristina Avonto	100%			
Post Doc	Sateesh Rotte	100%			
Post Doc	Vamshikrishna Manda	100%			
Post Doc	Prabhakar Peddikotla	100%			
Post Doc	Min Hye Yang	100%			
Post Doc	Naohito Abe	100%			
Post Doc	Satyanarayanaraju Sagi	100%			
Post Doc	Lu Lu	100%			
Assoc. R&D Biologist	Kavith Vijayasankar	50%			
R&D Botanist	Lal Jayaratna	100%			
Web Developer	Steven Hopper	100%			
Program Coordinator	Jennifer Taylor	100%			
Hourly Wages		100%			
Graduate Students (4)		100%			
Total Salaries and FB					\$1,241,865
equipment					\$150,000
supplies					\$188,902
travel					\$40,000
contractual services					\$120,000
MOBOT					\$54,296
Subtotal					\$1,795,063
F&A 44%					\$699,937
Total Request					\$2,495,000

BUDGET JUSTIFICATION

GRANT NUMBER
1U01FD004246

Provide a detailed budget justification for those line items and amounts that represent a significant change from that previously recommended. Use continuation pages if necessary.

A. PERSONNEL: \$1,241,856

Faculty and Professional Staff

PI, Dr. Ikhlas A. Khan, Assistant Director and Research Professor, National Center for Natural Products Research, Principal Investigator will devote 45% of his time to this program. Dr. Khan will be the contact point with NCNPR for interactions with FDA scientists. Dr. Khan has extensive research project administrative experience, has served as PI of several projects. Dr. Khan is currently working as a program director of FDA program at the Center. He works directly with Dr. Walker on a daily basis for scientific direction of major portions of NCNPR research efforts.

Co-Investigator, Dr. Larry A. Walker, Director, National Center for Natural Products Research, Co-Principal Investigator will devote 10% effort to the overall administrative direction of the program. Dr. Walker will organize and coordinate the Steering Committee operation, and work closely with FDA officials and University administration to ensure administrative compliance and performance. No costs will be incurred to the grant for Dr. Walker's support.

CURRENT BUDGET PERIOD

FROM
09/1/2012

THROUGH
08/31/2013

Explain any estimated unobligated balance (including prior year carryover) that is greater than 25% of the current year's total budget.
N/A

BUDGET JUSTIFICATION CONTINUATION

Faculty and Professional Staff Continued

Senior Research Scientist (Dr. Shabana I. Khan) - 23% effort. Dr. Khan will be responsible for direction of the efforts at UM to evaluate toxicological parameters for the natural products and botanical extracts. She will commit to the project. Dr. Khan's training is in biochemistry, and she has worked for many years in the screening development with natural products. She will supervise the efforts of the toxicology research associates.

Senior Research Scientist, (Dr. Troy Smillie) – 45% effort. Dr. Smillie will be responsible for all aspects of data management for the project. Dr. Smillie will coordinate particularly with the botanists, geneticist, analytical and isolation chemistry investigators, as well as with FDA scientists involved in the project, to develop and modify the data management workflow. Additionally Dr. Smillie is on the organizing committee for the Annual Oxford International Conference on the Science of Botanicals (www.oxfordICSB.org).

Research Scientist, Analytical Chemist. (Dr. Bharathi Avula) – 70% effort. Research Scientist, National Center for Natural Products Research will be responsible for analytical method development. Dr. Avula is a trained pharmacist and analytical chemist. She has developed expertise over the years in analysis of botanicals. She will also be supervising Dr.'s Yan Hong Wang, and Mei Wang in developing relevant analytical methods.

Senior Research Scientist, Analytical Chemist. (Dr. Yan Hong Wang) – 100% effort. Dr. Wang will be responsible for analytical method development. Dr. Wang is a natural products chemist with analytical background. He has developed an extensive expertise over the years in the analysis of botanicals.

Research Scientist, Plant Genetics (Dr. Natscha Techen) – 100% effort. Dr. Techen will be responsible for plant genetics approaches applied to the authentication of botanical dietary supplements. She is experienced in all the molecular techniques needed to accomplish this task.

Research Scientist, Isolation Chemist (Dr. Zulfiqar Ali) – 25% effort. Dr. Ali will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Research Scientist, Synthetic Chemist (Dr. Amar Chittiboyina) – 50% effort. Dr. Chittiboyina will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Research Scientist, Biologist (Dr. Gouyi Ma) – 100% effort. Dr. Ma will be responsible for the development of in-vitro assays to assess the toxicological profile of botanicals

Research Scientist, Chemist – (Ahmad Osman) 100% effort. This individual will be responsible for isolating and identifying compounds of interest from various botanical sources. Additionally, this person will be instrumental in compiling scientific information with regard to identification techniques (analytical, NMR, etc) for the authentication of botanical dietary supplements for the purpose of populating a dynamic web portal to assist FDA researchers and other stakeholders.

Associate Research Scientist, Isolation Chemist (Dr. Jiaping Zhao) – 53% effort. Dr. Zhao's responsibility will be to prepare extracts of selected botanicals, develop NMR identification/authentication techniques, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Postdoctoral Research Associate, Botanist (Dr. Vijayasankar Raman) – 100% effort. Dr. Raman will be working on macro and microscopy of botanicals, needed for species authentication. Microscopy provides an

understanding of anatomical features of a plant, which is instrumental in differentiating the species and also in determining potential adulterants.

Post Doctoral Research Associate, Analytical Chemist (Dr. Mei Wang) – 70% effort. Dr. Wang will develop analytical methods for the purpose of identifying botanical dietary supplement samples. Dr. Wang has several years of experience in developing analytical GC techniques for the purpose of analysis and quantitation.

Post Doctoral Research Associate, Chemist (Dr. Cristina Avonto) – 100% effort. Dr. Avonto will be responsible for isolating marker compounds and bioactive constituents from botanicals. Additionally Dr. Avonto will perform analytical profiling of botanicals using various GC techniques.

Post Doctoral Research Associate, Isolation Chemist (Dr. Sateesh Rotte) – 100% effort. Dr. Rotte will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Post Doctoral Research Associate, Biologist (Dr. Vamshikrishna Manda) – 100% effort. Dr. Manda will be responsible for the development of in-vitro assays to assess the safety of dietary supplement ingredients as well as ADMET evaluation of various constituents.

Post Doctoral Research Associate, Isolation Chemist (Dr. Prabhakar Peddikotla) – 100% effort. Dr. Peddikotla will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Post Doctoral Research Associate, Isolation Chemist (Dr. Min Hye Yang) – 100% effort. Dr. Yang will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Post Doctoral Research Associate, Analytical Chemist (Dr. Naohito Abe) – 100% effort. Dr. Abe will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Post Doctoral Research Associate, Isolation Chemist (Dr. Satyanarayanaraju Sagi) – 100% effort. Dr. Sagi will develop analytical methods for the purpose of identifying botanical dietary supplement samples. Dr. Sagi has several years of experience in developing analytical HPTLC/LC techniques for the purpose of analysis and quantitation.

Post Doctoral Research Associate, Isolation Chemist (Dr. Lu Lu) – 100% effort. Dr. Lu will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Assoc. R&D Biologist (Kavitha Vijayasankar) – 50% effort. This individual is responsible for the maintenance and inventory of the botanical repository as well as plant sourcing, sample processing and database cataloging of the materials associated for this center.

R&D Botanist (Lal Jayaratna) – 100% effort. This individual is responsible for maintaining the living collection of the centers medicinal plant garden. Additionally this individual prepares voucher specimens, maintains the programs seed bank and provides assistance/tours for training sessions and workshops.

Web Developer – (Steven Hopper) 100% effort. This individual will be responsible for the development of a botanical dietary supplement information portal for the dissemination of relevant scientific data and FDA Dietary Supplement cGMP information. In addition to this he will be aiding in the data collection/reporting efforts for this project.

Technical and Administrative Staff

Program Coordinator (Jennifer Taylor) - 100% effort. Ms. Taylor is responsible to the PIs, to allow for adequate follow-up with reports, publications, file documentation, sample tracking, etc. Ms. Taylor also provides vital logistical support for workshops, training sessions and conferences.

NOTE: The position of Program Coordinator is normally not allowed as direct costs under OMB circular A-21. However, we are requesting these direct costs be allowed due to the large scope of the project and the number of personnel to be managed and supported. This position is easily allocable to the project, and are reasonable given the size and nature of the project.

Hourly Wages – Hourly wage support (\$17,000) will be utilized for temporary and student workers employed in support of the project. These may be utilized in support of administrative or research activities, ranging from filing and data entry to field/greenhouse or laboratory assistance.

Graduate Students (4) – The graduate students will receive training in natural products chemistry, analytical chemistry. These students will be enrolled in the Ph.D. program in the Department of Pharmacognosy, or one of the other academic Departments in the school of Pharmacy (\$40,000).

Fringe Benefits

Fringe benefits for faculty and staff are calculated at the University's standard rate of 32.75% of salary. Fringe benefits for students (graduate or undergraduate) are calculated at the University's standard rate of 8% of wages.

Increase for additional Years:

Inflationary increases of 4% per year have been included for year for personnel positions

B. CONSULTANT COSTS: None

C. EQUIPMENT: \$150,000

These funds are to support the capacity for analytical work that is required for the success of the program. These funds will be utilized to either purchase new complete systems or to upgrade other existing equipment such as HPLC, GC, NMR or MS.

D. SUPPLIES: \$ 188,902

Primary commodity expenditures for the project will be for:

HPLC columns \$30,000

NMR/MS supplies (tubes, gases, columns) \$15,700

Microscopic supplies (slides, stains, optics, mounting preparation) \$6,700

Chromatographic supplies (TLC plates, chromatographic media, solvents) \$58,502

Mol. Biology supplies \$25,000

Botanical collection/storage materials \$15,000

Garden/greenhouse tools/supplies \$15,000

Books, databases other reference materials \$18,000

Computer supplies \$5,000

Total: \$ 188,902

E. TRAVEL: \$40,000

Included in this category are:

Travel for FDA Dietary Supplement GMP training sessions.

Establishing collection arrangements and collaborations (National and International)

Travel to/from Washington or other COE's for meetings with FDA officials

Travel costs related to hosting workshop/conference (National and International)

F. CONTRACTUAL SERVICES: \$120,000

Estimated expenditures in this category include contractual/professional services for:

Collection & documentation of plant material \$10,000
scale-up extraction/isolation \$8,000
taxonomic verifications \$6,000
maintenance contracts/repairs for analytical equipment \$20,000
software/upgrades for analytical equip. \$5,000
shipping, mailing costs \$4,000
Sub-Total: \$53,000

Estimated expenses for hosting conference:

Printing/PR \$5,000
Speaker reimbursements (28 @ 1,500) \$42,000
Dinners/breaks \$10,000
Staffing \$10,000
Sub Total: \$67,000

G. SUBCONTRACT: \$54,296

A subcontract with Missouri Botanical Garden will be in place in the amount of \$54,296. Missouri Botanical Garden will be collecting the specimens for this project and will be providing expertise on identification of the specimens. Dr. Rainer Bussmann, the director and curator of the William L. Brown Center for Plant Genetic Resource (WLBC), will be the Missouri Botanical Garden representative working on the project.

H. INDIRECT CHARGES: \$ 699,937

Indirect costs are calculated in accordance with The University of Mississippi's rate agreement with DHHS, dated September 12, 2011. Indirect costs for research are calculated at 44.0% of Total Direct Costs less equipment, tuition remission, and the portion of each sub-grant or subcontract in excess of \$25,000.

Program Director/Principal Investigator (Last, First, Middle): **Khan Ikhlas A**

PROGRESS REPORT SUMMARY	GRANT NUMBER 1U01FD004246	
	PERIOD COVERED BY THIS REPORT	
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Khan, Ikhlas, A	FROM 09/11/2012	THROUGH 08/31/2013

APPLICANT ORGANIZATION
The University of Mississippi

TITLE OF PROJECT (Repeat title shown in Item 1 on first page)
Science Based Authentication of Dietary Supplements

A. Human Subjects (Complete Item 6 on the Face Page)		
Involvement of Human Subjects	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change
B. Vertebrate Animals (Complete Item 7 on the Face Page)		
Use of Vertebrate Animals	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change
C. Select Agent Research	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change
D. Multiple PD/PI Leadership Plan	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change
E. Human Embryonic Stem Cell Line(s) Used	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

None

Progress Report Summary

A. Specific Aims

Under the provisions of the DSHEA, the FDA has primary responsibility for ensuring that appropriate regulatory actions are taken against marketed products that present significant health risks or bear false or misleading label claims. Foundational to the evaluation of such risks and claims is an adequate scientific base for decision-making. For botanical dietary supplements, development of such a science base is especially problematic because of several unique features, including the complexity of the constituents, variability of sourcing, lack of availability of reference materials, lack of manufacturing controls and rapidly expanding use in the marketplace. In 2010 the FDA fully implemented the current good manufacturing practices (cGMP) for the botanical dietary supplement industry placing the onus of “botanical identity and authenticity” on the manufacturers of botanical dietary supplements. However, these cGMP’s have in many ways increased the complexity as to what constitutes a “scientifically valid method” for the determination of the identity and authenticity of botanical materials. To address these complex issues surrounding botanical identity, authenticity, and safety and in continuation of our cooperative agreement with the FDA, the NCNPR has established the following specific aims to aid in the research needs of the FDA:

1. Assist in the identification and development of a list of BDS and botanical ingredients based on safety concerns, trends, and knowledge of botanicals being marketed in the U.S., to prioritize for further research.
2. Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to FDA, for evaluation of their safety.
3. Exchange technical and scientific information, analytical methods, and reference material with FDA scientists and other stakeholders.
4. Collaborate with FDA scientists in research areas of mutual interest.
5. Coordinate scientific workshops and conferences on BDS-related topics of public health relevance to address high priority science and research needs.

This renewal request expands the research under the current agreement on BDS to include potential safety issues, and extends the effort to include additional emerging problems associated with botanicals.

B. Studies and Results:

Aim 1: Assist in the identification and development of a list of BDS and botanical ingredients based on safety concerns, trends, and knowledge of botanicals being marketed in the U.S., to prioritize for further research.

Since the inception of the cooperative agreement between the NCNPR and the FDA in 2001, there have been numerous occasions for interactions between the two organizations. These have included (yearly) formal site visits by the FDA program officers, interactions during the annual International Conference on the Science of Botanicals (ICSB) and several conference calls and email exchanges. In addition to these interactions, over the past year the NCNPR has hosted five training sessions with the Office of Regulatory Affairs (ORA) to provide guidance to FDA inspectors for cGMP compliance issues for BDS’s (FD340). These training sessions have provided an opportunity for the programs project officer (Dr. Daniel Fabricant) to visit the NCNPR and stay abreast of the Centers ongoing developments. As always, researchers at the NCNPR are receptive to areas of study that are deemed necessary by the FDA for this project.

Aim 2: Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to FDA, for evaluation of their safety.

The availability of authenticated reference materials for botanicals is an essential first step in the development of analytical methodologies for the identification and quantitative analysis of botanical product(s). The NCNPR has been very successful in placing formal agreements with several international academic and governmental institutions including Pakistan, India (FRLHT), South Africa, Central/South America, China with the development of the Sino-US TCM Research Center and the established Center for Research in Indian Systems of Medicine (CRISM - www.CRISM.net) with the departments of AYUSH and CSIR in India. The

NCNPR has also established a Memorandum of Understanding (MOU) with the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Brazil and the Natural Products Utilization Research Unit (NPURU), USDA located in Oxford Mississippi. Lastly the NCNPR has cultivated a relationship with the Chinese FDA in order to obtain relevant authenticated Traditional Chinese Medicines (TCM's) and pertinent purified constituents for authentication purposes yielding approximately 50 new constituents over the past year.

From these and other collaborative relationships, the NCNPR has been able to acquire over 9500 plant samples and herbal extracts, representing approximately 4500 species over the duration of this project. There is a continuous effort in acquiring commercial samples as well for authentication purposes in addition to assuring that the developed identity testing techniques for this project are applicable for finished products in various formularies. Taking all these sample types into account there are over 15,000 samples within the NCNPR-FDA repository. All the plants and samples collected for this program have been assigned an inventory number and are cataloged in the NCNPR repository and voucher specimens of authentic samples are also maintained at the Thomas M. Pullen Herbarium in the Department of Biology at the University of Mississippi (MISSA).

In addition to this the NCNPR has a newly renovated Medicinal Plant Garden that maintains more than 270 species for selected growing (field, greenhouse and shade houses). The new facilities consist of two main buildings (4,362 sf. and 4,290 sf.) and four additional support buildings and structures sitting on approximately 5.25 acres of land. The Medicinal Plant Garden has developed significant collaborations with seed banks from 40 institutes from around the world in order to exchange medicinally important germplasms. This seed bank effort has yielded a collection of over 800 species to date. In addition, the garden personnel are preparing herbarium voucher specimens from the living collection and have started collecting samples for a DNA tissue bank. Over the course of this program the garden provided 320 authentic reference samples from the living collection for the Centers research efforts. The Medicinal Plant Garden is also registered under the Botanic Gardens Conservation International and is a member of the American Association of Botanical Gardens and Arboreta and International Plant Exchange Network (IPEN).

Isolation of reference compounds: The isolation of standard compounds is required to develop analytical methods, quantify individual compounds in extracts and to establish analytical fingerprints in support of quality and safety studies. Scientists at the NCNPR have isolated a number of compounds from species such as *Matricaria recutita* L., *Anthemis nobilis*, *Terminalia* spp., *Dioscorea villosa*, *Dioscorea cayenensis*, and *Lepidium meyenii* (Maca). It is through these continued efforts that the NCNPR scientist have isolated dozens of novel compounds as well as known analogs for analytical finger printing development for authentication purposes as well as safety analysis.

Analytical method development and metabolomic profiling: In the past year we have focused on developing several analytical methods for the determination of authenticity BDS's of interest. These studies include the development of UPLC (UV, ELS and MS), standard HPLC/HPTLC analytical methods as well as using proton NMR for metabolomic profiling for common botanicals including Cinnamon species (*Cinnamomum verum*, *C. cassia*, *C. loureiroi*, and *C. burmannii*), *Pausinystalia johimbe*, *Pausinystalia johimbe*, *Terminalia* spp. (*T. chebula*, *T. arjuna*, *T. bellirica*), *Phyllanthus emblica*, *Dioscorea cayennensis* Lam., *Dioscorea rotundata* Poir., *Dioscorea opposita* Thunb., *Dioscorea caucasica* Lipsky, *Dioscorea villosa* L., *Dioscorea bulbifera* L., *Dioscorea deltoidea* Wall. ex Griseb., *Dioscorea quaternata*, *Lepidium meyenii* (Maca), and *Euterpe oleracea* Mart. (acai berries). Most importantly, the Center aided in developing an analytical approach establishing the absence of dimethylamylamine (DMAA) in authenticated *Pelargonium graveolens*. This newly developed method provided the FDA with the information required to challenge the marketing of DMAA products for lack of safety evidence on April 27, 2012.

Aim 3: Exchange technical and scientific information, analytical methods, and reference material with FDA scientists and other stakeholders.

Dr. Ikhlas Khan, Scientific Director for the project, has been in touch regularly with Dr. Daniel Fabricant (CFSSAN, Director, Division of Dietary Supplement Programs), Dr. Elizabeth Calvey (CFSSAN liaison), Dr. Diego

Rua (CFSAN) and Dr. Robert L. Sprando, (CFSAN/OARSA, Director, Division of Toxicology) to exchange information on developed methods, reference materials availability, safety evaluations and project direction. These meetings and calls are designed to provide these individuals with an update of the NCNPR's overall program and to gain insight as to how this Center can better address the needs of the FDA.

Additionally, the NCNPR FDA-COE has also continued to assist the FDA's Office of Regulatory Affairs (ORA) in training inspectors for cGMP compliance for BDS's (FD340). Researchers at the NCNPR provided five one-day workshops on botanical dietary supplement authentication techniques to 193 trainees and FDA officials on December 12th, 2012, March 18th, 2013, April 22nd, 2013, May 20th, 2013, and June, 19th, 2013. The main training course is held in Memphis, Tennessee so that the trainees can attend a one-day excursion to the NCNPR for a combination of lectures and laboratory courses and training sessions to see what authentication techniques can be implemented for BDS's. The course covered current techniques utilized to identify botanical materials (Microscopy, Taxonomy, Macroscopy, TLC, HPLC, UPLC, GC, CE, etc) and was presented by Dr. Khan and colleagues at the NCNPR and included two one and one half hour lab courses on Analytical Methods, Botanical Authentication, and Nomenclature. It is anticipated that this training program will continue for the foreseeable future and will provide the FDA cGMP botanical dietary supplement inspectors a valuable tool that will assist in their ability to carry out their duties.

Researchers at the NCNPR have also provided their expertise in other training offered by the FDA/ORA/DHRD. One such course was an advanced level course for analysts who are performing regulatory sample analysis using mass spectrometry techniques for identification and authentication (LB 403). Specifically, Dr. Yan-Hong Wang provided a lecture to several FDA trainees on August 26th – 31st, 2012 covering the topic of how mass spectroscopy can be utilized for the authentication of botanicals. Dr. Wang also presented similar topics at a workshop organized by The University of Maryland and JIFSAN entitled "Dietary Supplements: Microscopic and Chemical Identification of Botanicals" on October the 15th-16th, 2012 to aid in the FDA's international food safety training program. As an extension of this joint training with JIFSAN, the NCNPR sent Dr. Suman Chandra to provide four presentations at a GAP & GMP workshop aimed at supply chain management for spices and botanical ingredients for the Indian spice board (September 17th-21st, 2012, Kochi, India). Dr. Chandra covered topics such as harvesting considerations, transportation/processing, cleaning and sanitation techniques. This workshop was then expanded into a multi-day multi-site visit for several members of the Indian spice board to provide these individuals with onsite training and lectures to provide advanced GAP and GMP techniques. This workshop, entitled "Food Safety and Supply Chain Management for Spices and Botanical Ingredients" was hosted by JIFSAN from March 25th - April 2nd then the NCNPR from April 3rd - 5th 2013.

Lastly, the research effort initiated by the establishment of this Center has provided several opportunities to leverage the existing cooperative agreement so as to expand the impact and overall benefit of the existing program. This includes a recently funded NCCAM/ODS Botanical Research Center grant to explore Botanical Estrogens: Mechanisms, Dose and Target Tissues (PD: Helferich, William G., University of Illinois/PL: Khan, Ikhlas, A., Award number 5 P50 AT006268-02). Under this grant the NCNPR is providing significant quantities and populations of authenticated samples of Licorice - *Glycyrrhiza glabra* Linné var *glabra*, Wild Yam - *Dioscorea villosa* L., and Dong Quai - *Angelica sinensis* (Oliv.) Diels for the established BRC. In addition to obtaining the outlined authenticated species for this program we have established a substantial growing and collection program for closely related species for authentication and comparative purposes. Additionally, the NCNPR has obtained funding from the USDA/ARS for a grant entitled Discovery and Development of Natural Product-Based Insect Management Compounds for Medicinal, Veterinary and Urban Concern, award number 58-6402-7-228. The purpose of this grant is to discover and develop new, safer and more effective insect management products derived from natural sources.

Aim 4: Collaborate with FDA scientists in research areas of mutual interest.

Over the past year the NCNPR has provided the FDA with scientific information for botanicals of concern as well as investigated several plants that have purported adverse events. Most notably we have undertaken an investigation of "chamomile" which has had reports of contact dermatitis and potential severe cases of allergic

reactions in cosmetic formulations. The initial question, as with all botanicals, is what is meant when discussing “chamomile” such as which species or variety was used, what plant part, what processing method was used, etc. As it turns out there are two main species of “chamomile” utilized for commerce within the United States German chamomile, *Matricaria recutita* L. and Roman chamomile, *Anthemis nobilis*. Typically the flowering tops are used for most cosmetic formulations and these are either added as powdered material or an extract (ethanolic, supercritical or steam distilled). Working closely with scientist in the FDA/CFSAN office of cosmetics and colors, we initiated an extraction and bioassay guided fractionation of both German and Roman chamomile utilizing an LLNA screening assay for lead identification. Initial results are indicated that there is a potential sensitizer within *Matricaria recutita* L. that could be causing the purported adverse events. Further investigation including isolation, purification and bioassay evaluations have been undertaken and are still in progress to identify the constituent(s) that contribute to the observed sensitization.

Additional investigations of cosmetic products that contain β -arbutin were also carried out. β -arbutin is a natural product that can be extracted from plants but can also be made synthetically. Cosmetics manufacturers are purportedly adding β -arbutin to products as a skin-whitening agent. However the EU Scientific Committee on Consumer Products (SCCP) determined in 2008 that β -arbutin in cosmetic products should be considered unsafe. In 2006, the FDA issued a notice of proposed rulemaking to establish that all skin bleaching products, whether marketed on a prescription or OTC basis, are drugs requiring an approved new drug application (NDA) for continued marketing. However the corresponding final rule has not been issued. The main safety concern for β -arbutin is that it can be a potential source for hydroquinone, which has displayed carcinogenicity in animals and poses a potential carcinogenic risk in humans. In addition, hydroquinone has been shown to cause disfiguring effects (ochronosis) after topical use at concentrations as low as 1 to 2-percent. Since β -arbutin can be extracted from plants, one general research objective was to seek further knowledge regarding which plants are best sources of β -arbutin such as *Salvia officinalis*. A developed analytical method was developed to differentiate between α -arbutin (synthetic) and β -arbutin (natural). This method was established and validated for plant sources that are known to contain arbutin, then utilized to analyze several (~30) botanical products that claimed to contain natural β -arbutin to see what levels of α and β arbutin each product contains. The results of this investigation showed that 52% and 30% products were found to contain β -arbutin and α -arbutin, respectively and that seven products did not contain β -arbutin or α -arbutin within the limits of detection. Stability testing of β -arbutin and α -arbutin indicated that while both compounds would degrade over time and under various conditions, they did not interconvert to the alternate form.

A second project undertaken for CFSAN's office of cosmetics and colors looked at products that include an essential oil known as “Tea tree oil”, which is obtained from several species of *Melaleuca* plants. One general research objective for this project is to seek further knowledge on compositional differences between commonly used species of *Melaleuca* plants (*Melaleuca alternifolia*, *Melaleuca linariifolia*, *Melaleuca viridiflora*, *Melaleuca leucadendron*, *Melaleuca dissitiflora*, etc.) in order to explore ways to address safety concerns for these species. The main safety concern about essential oils from these plant species is the potential for adverse effects on the skin, in particular sensitization or allergic contact dermatitis (ACD). It is not fully understood which of the tea tree oil constituents is responsible for ACD. Therefore, the potential ACD active(s) in tea tree oil are currently being identified utilizing an *in vitro* cell-based assay. Concurrently, we have developed an analytical GC/MS method to differentiate between the various species of tea tree oil that also identifies the major constituents. This newly developed method can be used to help identify potential ACD's within products.

Lastly, after extensive discussions with our FDA program officer, the Center established two in-house preliminary *in-vivo* safety evaluations for botanicals of interest. Priority evaluation was given to botanicals that have displayed an inherent risk based on literature information or reported adverse events. Initial screens focused on two areas of concern. The first mouse model measured botanicals for their potential to induce positive reinforcement or cause aversive properties using a conditioned place preference (CPP) paradigm procedure that is commonly used to evaluate drugs for their “addictive” behavior. Over the past year the Center used this model to evaluate *Salvia divinorum*, *Mitragyna speciosa* and fractions and pure compounds isolated from these species. For *S. divinorum* the preliminary results indicated that this particular botanical did not induce abusive potential. For *M. speciosa* the results indicated that the major pharmacologically active

constituent, mitragynine, has an abuse potential. The second *in-vivo* assay was developed to evaluate the potential hepatotoxicity's associated for botanical as measured in a mouse model where the hepatotoxicity is manifested by elevation of aminotransferases and histopathological features of acute hepatocellular necrosis and/or biliary injury. Initial *in-vivo* hepatotoxicity evaluations of EGCG, a major component in green tea products, at high doses can lead to mild liver injury and under febrile conditions it can cause severe liver injury. Both *in-vivo* models will continue to provide significant insight into the safety profile for botanicals that are of concern to public health.

Aim 5: Coordinate scientific workshops and conferences on BDS topics of public health relevance to address high priority science and research needs.

The Center hosted the 12th Oxford International Conference on the Science of Botanicals (ICSB) that was held on April 15th – 18th, 2013, at The University of Mississippi. This conference was co-sponsored by the Shanghai Institute of Materia Medica/CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant and Natural Products Research (GA); the Korean Society of Pharmacognosy and the Vietnam Academy of Science and Technology (VAST) the St Petersburg Institute of Pharmacy; the Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD) and the International Council on Medicinal and Aromatic Plants for Human Welfare (ICMAP) as such included representative delegations of scientists from various organizations in China, India and Europe. In addition to this there were representatives from several well-known international organizations as both attendees and presenters to total over 260 participants at this conference. The conference also included a specific session dedicated to highlight the scientific efforts of the established ODS/NCCAM Botanical Research Centers. The abstracts for this conference have been published in *Planta Medica*, 2013, 79(5), 369-421.

C. Significance:

Plant collection, authentication, voucher specimens, isolation of reference compounds and method development provide the basic tools for assessing the safety and quality of botanical products. Significant progress has been made by the NCNPR in each of these areas for several botanicals of interest over the past year. The acquired information is freely available to researchers at the FDA as well as physical samples (plants, extracts, etc) and phytochemical standards for evaluation purposes. Publications from this project have significantly contributed to the available methodological literature for the FDA and NCNPR (see references).

D. Plans:

Furthering the collaborative research effort we have established with CFSAN's office of cosmetics and colors, additional effort will continue to look at several potential areas of concern. The first being the continued investigation of cosmetic products that contain "arbutin"(s). The second project will entail continuing the exploration of products that contain "tea tree" essential oil(s) which can be derived from several species of *Melaleuca* plants. The main research objective for this project will be to seek further knowledge on compositional differences between commonly used species of *Melaleuca* plants (*Melaleuca alternifolia*, *Melaleuca linariifolia*, *Melaleuca viridiflora*, *Melaleuca leucadendron*, *Melaleuca dissitiflora*, etc.) in order to explore ways to address safety concerns for these species and their potential for producing ACD activity.

Continual research effort will also focus on the two recently developed in-house *in-vivo* screen evaluating botanicals for their potential to induce positive reinforcement or cause aversive properties using the developed CPP paradigm procedure that is commonly used to evaluate drugs for "addictive" behavior and the second assay which evaluates potential hepatotoxicity associated for certain botanicals. Priority evaluation will be given to botanicals that have displayed an inherent risk based on literature information or reported adverse events. Both models will provide significant insight as to the safety profile for botanicals that are of concern to the public health.

Lastly, expansion of the existing repository of reference plant samples for the genera noted throughout this report will be a continuing priority for this project. New botanicals will be selected based on input received from the FDA program officer, collaborators and liaison for further studies and to evaluate their safety and quality. A

thirteenth and fourteenth conferences are being organized. The first will be a shorter workshop entitled Oxford International Conference on the Science of Botanicals (ICSB) and will be held on April 14th – 15^h, 2014 and the second will be a full conference that will be held in conjunction with the American Society of Pharmacognosy's 2014 Annual meeting on August 2nd-6th, 2014. These conferences will be co-sponsored by the Shanghai Institute of Materia Medica/CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the Ministry of Indigenous Medicine, Sri Lanka; the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant and Natural Products Research (GA); the Korean Society of Pharmacognosy and the Vietnam Academy of Science and Technology (VAST) the St Petersburg Institute of Pharmacy; the Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD) and the International Council on Medicinal and Aromatic Plants for Human Welfare (ICMAP). This conference will cover the topics of TCM, Ayurveda, cultivation, collection, post-harvest, processing practices, identification, authentication and quality/safety assessment of botanicals including current clinical evaluations, regulatory aspects and the impact on the global dietary supplement industry. Again the proceedings from these conferences are expected to be published in *Planta Medica*.

References

1. Wang, Y.-H.; Avula, B.; Nanayakkara, N. P. D.; Zhao, J.; Khan, I. A., Cassia Cinnamon as a Source of Coumarin in Cinnamon-Flavored Food and Food Supplements in the United States. *Journal of Agricultural and Food Chemistry* **2013**, 61, (18), 4470-4476.
2. Raman, V.; Avula, B.; Galal, A. M.; Wang, Y.-H.; Khan, I. A., Microscopic and UPLC-UV-MS analyses of authentic and commercial yohimbe (*Pausinystalia johimbe*) bark samples. *J. Nat. Med.* **2013**, 67, 42-50.
3. Haron, M. H.; Avula, B.; Khan, I. A.; Mathur, S. K.; Dasmahapatra, A. K., Modulation of ethanol toxicity by *Asian ginseng* (*Panax ginseng*) in Japanese ricefish (*Oryzias latipes*) embryogenesis. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **2013**, 157, (3), 287-297.
4. Hapuarachchi, S. D.; Ali, Z.; Abe, N.; Sugandhika, S. T.; Sandun, S. T.; Khan, I. A., Andrographidine G, a new flavone glucoside from *Andrographis paniculata*. *Natural product communications* **2013**, 8, (3), 333-334.
5. Avula, B.; Wang, Y.-H.; Wang, M.; Shen, Y.-H.; Khan, I. A., Simultaneous Determination and Characterization of Tannins and Triterpene Saponins from the Fruits of Various Species of Terminalia and Phyllanthus emblica Using a UHPLC-UV-MS Method: Application to Triphala. *Planta Medica* **2013**, 79, (02), 181-188.
6. Ali, Z.; Smillie, T. J.; Khan, I. A., Two spirostan steroid glycoside fatty esters from *Dioscorea cayenensis*. *Natural product communications* **2013**, 8, (3), 323-326.
7. Ali, Z.; Smillie, T. J.; Khan, I. A., Cholestane steroid glycosides from the rhizomes of *Dioscorea villosa* (wild yam). *Carbohydrate Research* **2013**, 370, (0), 86-91.
8. Zhao, J.; Avula, B.; Chan, M.; Clément, C.; Kreuzer, M.; Khan, I. A., Metabolomic Differentiation of Maca (*Lepidium meyenii*) Accessions Cultivated under Different Conditions Using NMR and Chemometric Analysis. *Planta Medica* **2012**, 78, (01), 90-101.
9. Wang, Y.-H.; Avula, B.; Fu, X.; Wang, M.; Khan, I. A., Simultaneous Determination of the Absolute Configuration of Twelve Monosaccharide Enantiomers from Natural Products in a Single Injection by a UPLC-UV/MS Method. *Planta Med* **2012**, 78, 834-837.
10. Rumalla, C. S.; Avula, B.; Wang, Y.-H.; Smillie, T. J.; Khan, I. A., Densitometric-HPTLC method development and analysis of anthocyanins from acai (*Euterpe oleracea* Mart.) berries and commercial products. *JPC. Journal of planar chromatography, modern TLC* **2012**, 25, (5), 409-414.
11. ElSohly, M. A.; Gul, W.; ElSohly, K. M.; Murphy, T. P.; Weerasooriya, A.; Chittiboyina, A. G.; Avula, B.; Khan, I. A.; Eichner, A.; Bowers, L. D., Pelargonium Oil and Methyl Hexaneamine (MHA): Analytical Approaches Supporting the Absence of MHA in Authenticated *Pelargonium graveolens* Plant Material and Oil. *Journal of Analytical Toxicology* **2012**.

ALL PERSONNEL REPORT

GRANT NUMBER

U01 FD004246

Place this form at the end of the signed original copy of the application. Do not duplicate.

Always list the PD/PI(s). In addition, list all other personnel who participated in the project during the current budget period for at least one person month or more, regardless of the source of compensation (a person month equals approximately 160 hours or 8.3% of annualized effort). Use the following abbreviated categories for describing Role on Project:

- PD/PI
- Co-Investigator
- Faculty Collaborator
- Staff Scientist (doctoral level)
- Postdoc (Postdoctoral Scholar, Fellow, or Other Postdoctoral Position)
- Grad Rsch Asst (Graduate Research Assistant)
- Undergrad Rsch Asst (Undergraduate Research Assistant)
- Rsch Asst (Research Assistant/Coordinator)
- Technician
- Consultant
- Biostatistician
- Other (Specify)

If personnel are supported by a Reentry or Diversity Supplement or American Recovery and Reinvestment Act (ARRA) funding, please indicate such after the Role on Project, using the following abbreviations: RS - Reentry Supplement; DS - Diversity Supplement; AF - General ARRA Supplement; ASE - ARRA Summer Experience funding.

Use Cal (calendar), Acad, or Summer to enter months devoted to project.

Commons ID	Name	Degree(s)	SSN (last 4 digits)	Role on Project	DoB (MM /YY)	Cal	Acad	Summer
IKHLAS	Ikhlas A. Khan	Ph.D.	(b) (6)	PI	(b) (6)	5.4		
LARRYWA LKER2004	Larry A. Walker	Ph.D.		Co-PI		1.2		
SKHAN	Shabana Khan	Ph.D.		Staff Scientist		2.76		
TSMILLIE	Troy Smillie	Ph.D.		Staff Scientist		5.4		
BAVULA	Bharathi Avula	Ph.D.		Staff Scientist		8.4		
YAN HONG	Yan Hong Wang	Ph.D.		Staff Scientist		12		
	Natascha Techen	Ph.D.		Staff Scientist		12		
ALI	Zulfiqar Ali	Ph.D.		Staff Scientist		3		
CHITTIBOY INA	Amar Chittiboyina	Ph.D.		Staff Scientist		6		
	Gouyi Ma	Ph.D.		Staff Scientist		12		
	Ahmad Osman	Ph.D.		Staff Scientist		12		
JPZHAO	Jianping Zhou	Ph.D.		Staff Scientist		6.36		
VRAMAN	Vijayasankar Raman	Ph.D.		Postdoc		12		
	Mei Wang	Ph.D.		Postdoc		8.4		

ALL PERSONNEL REPORT

GRANT NUMBER

U01 FD004246

Place this form at the end of the signed original copy of the application. Do not duplicate.

Always list the PD/PI(s). In addition, list all other personnel who participated in the project during the current budget period for at least one person month or more, regardless of the source of compensation (a person month equals approximately 160 hours or 8.3% of annualized effort). Use the following abbreviated categories for describing Role on Project:

- PD/PI
- Co-Investigator
- Faculty Collaborator
- Staff Scientist (doctoral level)
- Postdoc (Postdoctoral Scholar, Fellow, or Other Postdoctoral Position)
- Grad Rsch Asst (Graduate Research Assistant)
- Undergrad Rsch Asst (Undergraduate Research Assistant)
- Rsch Asst (Research Assistant/Coordinator)
- Technician
- Consultant
- Biostatistician
- Other (Specify)

If personnel are supported by a Reentry or Diversity Supplement or American Recovery and Reinvestment Act (ARRA) funding, please indicate such after the Role on Project, using the following abbreviations: RS - Reentry Supplement; DS - Diversity Supplement; AF - General ARRA Supplement; ASE - ARRA Summer Experience funding.

Use Cal (calendar), Acad, or Summer to enter months devoted to project.

Commons ID	Name	Degree(s)	SSN (last 4 digits)	Role on Project	DoB (MM /YY)	Cal	Acad	Summer
	Christina Avonto	Ph.D.	(b) (6)	Postdoc	(b) (6)	12		
	Sateesh Rotte	Ph.D.		Postdoc		12		
	Vamshikrishna Manda	Ph.D.		Postdoc		12		
	Prabhakar Peddikotla	Ph.D.		Postdoc		12		
	Min Hye Yang	Ph.D.		Postdoc		12		
	Naohito Abe	Ph.D.		Postdoc		12		
	Satyanarayanaraju Sagi	Ph.D.		Postdoc		12		
	Lu Lu	Ph.D.		Postdoc		12		
	Kavitha Vijayasankar	MSc		Rsch Asst		6		
	Lal Jayaratna	MSc		Rsch Asst		12		
	Steven Hopper	BFA		Technician		12		
	Jennifer Taylor			Rsch Asst		12		

THE UNIVERSITY OF MISSISSIPPI INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
ANIMAL STUDY APPLICATION APPROVAL

Application No.: 12-020

Approval Date: 06-20-12

Project Expiration Date: 06-20-15

Approved Pain Category: A

Animals to be Used & No. Approved: 2700 Rats for 3 year approval period

TO: Dr. Sufka
Department of Psychology

FROM: Jennifer Caldwell, Ph.D.
IACUC Research Compliance Specialist

SUBJECT: Protocol 12-020, Screening botanical compounds for rewarding/aversive properties

Your application was reviewed by the Institutional Animal Care and Use Committee (IACUC) and determined appropriate for designated review. The assigned designated reviewers completed their review and approved protocol 12-020 on 06-20-12.

Regulations require that you:

- 1. Post a copy of this approval letter in your animal room for the three-year IACUC approval period. (PHS Policy IV, D, a-e; AWAR §2.35, a, 2, and 3)**
- 2. Submit a renewal application to continue this study beyond the three-year approval period. (PHS Policy IV, C. 1 – IV, C. 4) (We will remind you well before the 3-year expiration date.)**

In order to maintain your approval status, you must submit an annual report of progress on this study (AWAR §2.31, d, 5 and PHS Policy IV, C, 5) using a Protocol Annual Update form. (We will send this form to you electronically several months before the due date.)

Animal purchase requisitions for labs located in the University Vivarium must be submitted to Ms. Penni Bolton, Animal Facility Supervisor.

Procedure, personnel, and other changes must be first approved by the Committee. Complete one of the following forms:

- Non-Personnel Protocol Amendment
- Protocol Amendment for Personnel Changes Only

Approval for adding personnel requires that the new person(s):

- Complete required Health & Safety and species-specific training BEFORE engaging in any activity involving live vertebrate animals.
- If conducting surgery, complete mandatory training and performance of surgical techniques in the procedure(s) and species indicated. Must receive surgery proficiency certification from the Attending Veterinarian (Dr. Fyke).
- Submit the Occupational Health Evaluation and OHSP Risk Inventory forms to the Attending Veterinarian (Dr. Fyke, B104 NCNPR) for review and approval by the Occupational Health Physician.
- Read the materials on asthma and allergy for animal handlers.
- Complete the AALAS Learning Library online course "Laboratory Animal Allergy."
- Check to make sure tetanus vaccination is current. The Centers for Disease Control and Prevention recommends tetanus vaccinations every 10 years, and the IACUC strongly urges personnel to get this free vaccination. (The ORSP Division of Research Integrity and Compliance pays the \$45.00 fee.).

Please contact the IACUC office staff at 915-7482 or askiacuc@olemiss.edu if you need assistance or have any questions.

cc: Ms. Penni Bolton, Animal Facility Supervisor

THE UNIVERSITY OF MISSISSIPPI INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
ANIMAL STUDY APPLICATION APPROVAL

Application No.: 13-007

Approval Date: 10-31-12

Project Expiration Date: 10-31-15

Approved Pain Category: B

Animals to be Used & No. Approved: 2410 Mice for 3 year approval period

TO: Dr. Ashfaq
Department of NCNPR

FROM: Jennifer Caldwell, Ph.D.
IACUC Research Compliance Specialist

SUBJECT: Protocol 13-007, Evaluation of Hepatotoxicity of Botanical Supplements

Your application was reviewed by the Institutional Animal Care and Use Committee (IACUC) and determined appropriate for designated review. The assigned designated reviewers completed their review and approved protocol 13-007 on 10-31-12.

Regulations require that you:

- 1. Post a copy of this approval letter in your animal room for the three-year IACUC approval period. (PHS Policy IV, D, a-e; AWAR §2.35, a, 2, and 3)**
- 2. Submit a renewal application to continue this study beyond the three-year approval period. (PHS Policy IV. C. 1 – IV. C. 4) (We will remind you well before the 3-year expiration date.)**

In order to maintain your approval status, you must submit an annual report of progress on this study (AWAR §2.31, d, 5 and PHS Policy IV, C, 5) using a Protocol Annual Update form. (We will send this form to you electronically several months before the due date.)

Animal purchase requisitions for labs located in the University Vivarium must be submitted to Ms. Penni Bolton, Animal Facility Supervisor.

Procedure, personnel, and other changes must be first approved by the Committee. Complete one of the following forms:

- Non-Personnel Protocol Amendment
- Protocol Amendment for Personnel Changes Only

Approval for adding personnel requires that the new person(s):

- Complete required Health & Safety and species-specific training **BEFORE** engaging in any activity involving live vertebrate animals.
- If conducting surgery, complete mandatory training and performance of surgical techniques in the procedure(s) and species indicated. Must receive surgery proficiency certification from the Attending Veterinarian (Dr. Fyke).
- Submit the Occupational Health Evaluation and OHSP Risk Inventory forms to the Attending Veterinarian (Dr. Fyke, B104 NCNPR) for review and approval by the Occupational Health Physician.
- Read the materials on asthma and allergy for animal handlers.
- Complete the AALAS Learning Library online course "Laboratory Animal Allergy."
- Check to make sure tetanus vaccination is current. The Centers for Disease Control and Prevention recommends tetanus vaccinations every 10 years, and the IACUC strongly urges personnel to get this free vaccination. (The ORSP Division of Research Integrity and Compliance pays the \$45.00 fee.)

Please contact the IACUC office staff at 915-7482 or _____ if you need assistance or have any questions.


cc: Ms. Penni Bolton, Animal Facility Supervisor

Department of Health and Human Services
Public Health Services

Review Group	Type	Activity 5	Grant Number 1U01FD004246 - 04
Total Project Period			
From: 09/15/2011		Through: 08/31/2011	
Requested Budget Period			
From: 09/1/2014		Through: 08/31/2015	

Grant Progress Report

1. TITLE OF PROJECT Science Based Authentication of Dietary Supplements			
2a. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR (Name and address, street, city, state, zip code) Khan, Ikhlas A. 120 Faser Hall/NCNPR School of Pharmacy University of Mississippi University, MS 38677		2b. E-MAIL ADDRESS ikhlan@olemiss.edu	
		2c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT National Center for Natural Products Research	
		2d. MAJOR SUBDIVISION School of Pharmacy	
		2e. Tel: 662-915-7821 Fax: 662-915-7989	
3a. APPLICANT ORGANIZATION (Name and address, street, city, state, zip code) The University of Mississippi Office of Research and Sponsored Programs 100 Barr Hall PO Box 907 University, MS 38677		3b. Tel: 662-915-7482 Fax: 662-915-7577	
		3c. DUNS: 067713560	
		4. ENTITY IDENTIFICATION NUMBER 1646001159A1	
6. HUMAN SUBJECTS <input checked="" type="checkbox"/> No <input type="checkbox"/> Yes		5. NAME, TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL	
6a. Research Exempt <input checked="" type="checkbox"/> No <input type="checkbox"/> Yes		Mickey McClaurin, Director of Sponsored Programs Office of Research and Sponsored Programs 100 Barr Hall or PO Box 907, University MS 38677	
If Exempt ("Yes" in 6a): Exemption No.		Tel: 662-915-7482 Fax: 662-915-7577	
If Not Exempt ("No" in 6a): IRB approval date		E-MAIL: research@olemiss.edu	
6b. Federal Wide Assurance No.			
6c. NIH-Defined Phase III Clinical Trial <input checked="" type="checkbox"/> No <input type="checkbox"/> Yes			
7. VERTEBRATE ANIMALS <input type="checkbox"/> No <input checked="" type="checkbox"/> Yes		10. PROJECT/PERFORMANCE SITE(S)	
7a. If "Yes," IACUC approval Date 06-20-12		Organizational Name: University of Mississippi	
7b. Animal Welfare Assurance No. A3356-01		DUNS: 067713560	
8. COSTS REQUESTED FOR NEXT BUDGET PERIOD		Street 1: 120 Faser Hall/NCNPR	
8a. DIRECT \$1,798,535		Street 2: School of Pharmacy	
8b. TOTAL \$2,500,000			
9. INVENTIONS AND PATENTS <input checked="" type="checkbox"/> No <input type="checkbox"/> Yes		City: University	
If "Yes," <input type="checkbox"/> Previously Reported <input type="checkbox"/> Not Previously Reported		County: Lafayette	
		State: MS	
		Province:	
		Country: USA	
		Zip/Postal Code: 38677	
		Congressional Districts: MS-001	
11. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Item 13) Mickey McClaurin, Director of Sponsored Programs Administration			
TEL: 662-915-7482		FAX: 662-915-7577	
		E-MAIL: research@olemiss.edu	
12. Corrections to Page 1 Face Page			

13. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.	SIGNATURE OF OFFICIAL NAMED IN 11. (In ink) 	DATE 5/30/14
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			Salary	Fringe	Total
Personnel					
PI	Ikhlas Khan	45%	(b) (6)		
Co-PI	Larry Walker				
Principle Res. Scientist	Shabana Khan	23%			
Post Doc	Amira Wanas	50%			
Sr. Research Scientist	Bharthi Avula	70%			
Sr. Research Scientist	Yan Hong Wang	100%			
Res. Scientist	Natascha Tehen	100%			
Res. Scientist	Zulfiqar Ali	25%			
Sr. Research Scientist	Amar Chittiboyina	50%			
Res. Scientist	Guoyi Ma	100%			
Res. Scientist	Ahmad Osman	100%			
Assoc. Res. Scientist	Jianping Zhou	53%			
Post Doc	Vijayasankar Raman	100%			
Post Doc	Mei Wang	70%			
Post Doc	Cristina Avonto	100%			
Post Doc	Vamshikrishna Manda	100%			
Post Doc	Zhihao Zhang	100%			
Post Doc	Min Hye Yang	100%			
Assoc. R&D Biologist	Helaina Craig	100%			
	Satyanarayanaraju Sagi	100%			
Post Doc	Pradeep Lasonkar	100%			
Post Doc	Iffat Parveen	100%			
R&D Botanist	Lal Jayaratna	100%			
R&D Data Analyst	Steven Hopper	100%			
Project Coordinantor	Gray Dale	25%			
Program Coordinator	Jennifer Taylor	100%			
Hourly Wages		100%			
Graduate Students (4)		100%			
Total Salaries and FB					\$1,241,419
equipment					\$150,000
supplies					\$165,181
travel					\$40,000
contractual services					\$140,000
MOBOT					\$54,296
Subtotal					\$1,790,896
F&A 44%					\$709,104
Total Request					\$2,500,000

Program Director/Principal Investigator (Last, First, Middle): Khan, Ikhlas, A.

BUDGET JUSTIFICATION

GRANT NUMBER
1U01FD004246

Provide a detailed budget justification for those line items and amounts that represent a significant change from that previously recommended. Use continuation pages if necessary.

A. PERSONNEL: \$1,241,419

Faculty and Professional Staff

PI, Dr. Ikhlas A. Khan, Assistant Director and Research Professor, National Center for Natural Products Research, Principal Investigator will devote 45% of his time to this program. Dr. Khan will be the contact point with NCNPR for interactions with FDA scientists. Dr. Khan has extensive research project administrative experience, has served as PI of several projects. Dr. Khan is currently working as a program director of FDA program at the Center. He works directly with Dr. Walker on a daily basis for scientific direction of major portions of NCNPR research efforts.

Co-Investigator, Dr. Larry A. Walker, Director, National Center for Natural Products Research, Co-Principal Investigator will provide the time & effort necessary for the overall administrative direction of the program. Dr. Walker will organize and coordinate the Steering Committee operation, and work closely with FDA officials and University administration to ensure administrative compliance and performance. No costs will be incurred to the grant for Dr. Walker's support.

CURRENT BUDGET PERIOD

FROM
09/01/13

THROUGH
08/31/14

Explain any estimated unobligated balance (including prior year carryover) that is greater than 25% of the current year's total budget.
N/A

BUDGET JUSTIFICATION CONTINUATION

Faculty and Professional Staff Continued

Principle Research Scientist (Dr. Shabana I. Khan) - 23% effort. Dr. Khan will be responsible for direction of the efforts at UM to evaluate toxicological parameters for the natural products and botanical extracts. She will commit to the project. Dr. Khan's training is in biochemistry, and she has worked for many years in the screening development with natural products. She will supervise the efforts of the toxicology research associates.

Sr. Research Scientist, Synthetic Chemist (Dr. Amar Chittiboyina) – 50% effort. Dr. Chittiboyina will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards. Dr. Chittiboyina will be responsible for all scientific aspects of data management for the project. Dr. Chittiboyina will coordinate particularly with the botanists, geneticist, analytical and isolation chemistry investigators, as well as with FDA scientists involved in the project, to develop and modify the data management workflow.

Sr. Research Scientist, Analytical Chemist. (Dr. Bharathi Avula) – 70% effort. Research Scientist, National Center for Natural Products Research will be responsible for analytical method development. Dr. Avula is a trained pharmacist and analytical chemist. She has developed expertise over the years in analysis of botanicals. She will also be supervising Dr.'s Yan Hong Wang, and Mei Wang in developing relevant analytical methods.

Senior Research Scientist, Analytical Chemist. (Dr. Yan Hong Wang) – 100% effort. Dr. Wang will be responsible for analytical method development. Dr. Wang is a natural products chemist with analytical background. He has developed an extensive expertise over the years in the analysis of botanicals.

Research Scientist, Plant Genetics (Dr. Natscha Tehen) – 100% effort. Dr. Tehen will be responsible for plant genetics approaches applied to the authentication of botanical dietary supplements. She is experienced in all the molecular techniques needed to accomplish this task.

Research Scientist, Isolation Chemist (Dr. Zulfiqar Ali) – 25% effort. Dr. Ali will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Research Scientist, Biologist (Dr. Gouyi Ma) – 100% effort. Dr. Ma will be responsible for the development of in-vitro assays to assess the toxicological profile of botanicals

Research Scientist, Chemist – (Ahmad Osman) 100% effort. This individual will be responsible for isolating and identifying compounds of interest from various botanical sources. Additionally, this person will be instrumental in compiling scientific information with regard to identification techniques (analytical, NMR, etc) for the authentication of botanical dietary supplements for the purpose of populating a dynamic web portal to assist FDA researchers and other stakeholders.

Associate Research Scientist, Isolation Chemist (Dr. Jiaping Zhao) – 53% effort. Dr. Zhao's responsibility will be to prepare extracts of selected botanicals, develop NMR identification/authentication techniques, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Postdoctoral Research Associate, Botanist (Dr. Vijayasankar Raman) – 100% effort. Dr. Raman will be working on macro and microscopy of botanicals, needed for species authentication. Microscopy provides an understanding of anatomical features of a plant, which is instrumental in differentiating the species and also in determining potential adulterants.

Program Director/Principal Investigator (Last, First, Middle): Khan, Ikhlas, A.

Post Doctoral Research Associate, Analytical Chemist (Dr. Mei Wang) – 70% effort. Dr. Wang will develop analytical methods for the purpose of identifying botanical dietary supplement samples. Dr. Wang has several years of experience in developing analytical GC techniques for the purpose of analysis and quantitation.

Post Doctoral Research Associate, Chemist (Dr. Cristina Avonto) – 100% effort. Dr. Avonto will be responsible for isolating marker compounds and bioactive constituents from botanicals. Additionally Dr. Avonto will perform analytical profiling of botanicals using various GC techniques.

Post Doctoral Research Associate, Biologist (Dr. Vamshikrishna Manda) – 100% effort. Dr. Manda will be responsible for the development of in-vitro assays to assess the safety of dietary supplement ingredients as well as ADMET evaluation of various constituents.

Post Doctoral Research Associate, Isolation Chemist (Zhihao Zhang) – 100% effort. Dr. Zhang will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Post Doctoral Research Associate, Isolation Chemist (Dr. Min Hye Yang) – 100% effort. Dr. Yang will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Assoc. R&D Biologist (Helaina Craig) – 100% effort. Ms. Craig will help the senior scientists on animal based in vivo work for behavioral and hepatotoxic studies on the botanical of interest.

Post Doctoral Research Associate, Isolation Chemist (Dr. Satyanarayanaraju Sagi) – 100% effort. Dr. Sagi will develop analytical methods for the purpose of identifying botanical dietary supplement samples. Dr. Sagi has several years of experience in developing analytical HPTLC/LC techniques for the purpose of analysis and quantitation.

Post Doctoral Research Associate, Isolation Chemist (Dr. Pradeep Lasonkar) – 100% effort. Dr. Lasonkar will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Post Doctoral Research Associate, Plant Genetics (Iffat Parveen) – 100% effort. Dr. Parveen will be responsible for plant genetics approaches applied to the authentication of botanical dietary supplements. She will assist and train under Dr. Tehen for the molecular techniques needed to accomplish the proposed work.

Post Doctoral Research Associate, Analytical Chemist (Amira Wanas) – 50% effort. Dr. Wanas will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

R&D Botanist (Lal Jayaratna) – 100% effort. This individual is responsible for maintaining the living collection of the centers medicinal plant garden. Additionally this individual prepares voucher specimens, maintains the programs seed bank and provides assistance/tours for training sessions and workshops.

R&D Data Analyst – (Steven Hopper) 100% effort. This individual will be responsible for the development of a botanical dietary supplement information portal for the dissemination of relevant scientific data and FDA Dietary Supplement cGMP information. In addition to this he will be aiding in the data labeling, collection/reporting efforts for this project.

Project Coordinator (Gray Dale) - 25% effort. Mr. Dale is responsible to the PIs, to allow for adequate follow-up with reports, budgets and travel. Mr. Dale also provides vital logistical support for ICSB conference.

Technical and Administrative Staff

Program Coordinator (Jennifer Taylor) - 100% effort. Ms. Taylor is responsible to the PIs, to allow for adequate follow-up with reports, publications, file documentation, sample tracking, etc. Ms. Taylor also provides vital logistical support for workshops, training sessions and conferences.

NOTE: The position of Program Coordinator and Project Coordinator is normally not allowed as direct costs under OMB circular A-21. However, we are requesting these direct costs be allowed due to the large scope of the project and the number of personnel to be managed and supported. This position is easily allocable to the project, and are reasonable given the size and nature of the project.

Hourly Wages – Hourly wage support (\$17,000) will be utilized for temporary and student workers employed in support of the project. These may be utilized in support of administrative or research activities, ranging from filing and data entry to field/greenhouse or laboratory assistance.

Graduate Students (4) – The graduate students will receive training in natural products chemistry, analytical chemistry. These students will be enrolled in the Ph.D. program in the Department of Pharmacognosy, or one of the other academic Departments in the school of Pharmacy (\$40,000).

Fringe Benefits

Fringe benefits for faculty and staff are calculated at the University's standard rate of 32.75% of salary. Fringe benefits for students (graduate or undergraduate) are calculated at the University's standard rate of 8% of wages.

Increase for additional Years:

Inflationary increases of 3% per year have been included for year for personnel positions

B. CONSULTANT COSTS: None

C. EQUIPMENT: \$150,000

These funds are to support the capacity for analytical work that is required for the success of the program. These funds will be utilized to either purchase new complete systems or to upgrade other existing equipment such as HPLC, GC, NMR or MS.

D. SUPPLIES: \$ 165,181

Primary commodity expenditures for the project will be for:

HPLC columns \$22,947

NMR/MS supplies (tubes, gases, columns) \$13,700

Microscopic supplies (slides, stains, optics, mounting preparation) \$6,700

Chromatographic supplies (TLC plates, chromatographic media, solvents) \$50,834

Mol. Biology supplies \$30,000

Botanical collection/storage materials \$13,000

Garden/greenhouse tools/supplies \$12,000

Books, databases other reference materials \$12,000

Computer supplies \$4,000

Sub Total: \$165,181

E. TRAVEL: \$40,000

Included in this category are:

Travel for FDA Dietary Supplement GMP training sessions.

Establishing collection arrangements and collaborations (National and International)

Travel to/from Washington or other COE's for meetings with FDA officials

Travel costs related to hosting workshop/conference (National and International)

F. CONTRACTUAL SERVICES: \$140,000

Estimated expenditures in this category include contractual/professional services for:

Collection & documentation of plant material \$9,000

scale-up extraction/isolation \$ 9,000

taxonomic verifications \$6,000

maintenance contracts/repairs for analytical equipment \$39,500

software/upgrades for analytical equip. \$8,000

shipping, mailing costs \$4,000

Sub-Total: \$75,500

Estimated expenses for hosting conference:

Printing/PR \$3,500

Speaker reimbursements (28 @ 1,500) \$42,000

Dinners/breaks \$11,000

Staffing \$8,000

Sub Total: \$64,500

G. SUBCONTRACT: \$54,296

A subcontract with Missouri Botanical Garden will be in place in the amount of \$54,296. Missouri Botanical Garden will be collecting the specimens for this project and will be providing expertise on identification of the specimens. Dr. Rainer Bussmann, the director and curator of the William L. Brown Center for Plant Genetic Resource (WLBC), will be the Missouri Botanical Garden representative working on the project.

H. INDIRECT CHARGES: \$ 709,104

Indirect costs are calculated in accordance with The University of Mississippi's rate agreement with DHHS, dated September 12, 2011. Indirect costs for research are calculated at 44.0% of Total Direct Costs less equipment, tuition remission, and the portion of each sub-grant or subcontract in excess of \$25,000.

Program Director/Principal Investigator (Last, First, Middle): Khan, Ikhlas A

PROGRESS REPORT SUMMARY	GRANT NUMBER 1U01FD004246	
	PERIOD COVERED BY THIS REPORT	
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Khan, Ikhlas A	FROM 09/1/2013	THROUGH 08/31/2014
APPLICANT ORGANIZATION The University of Mississippi		
TITLE OF PROJECT (Repeat title shown in Item 1 on first page) Science Based Authentication of Dietary Supplements		

A. Human Subjects (Complete Item 6 on the Face Page)

Involvement of Human Subjects ☒ No Change Since Previous Submission ☐ Change

B. Vertebrate Animals (Complete Item 7 on the Face Page)

Use of Vertebrate Animals ☒ No Change Since Previous Submission ☐ Change

C. Select Agent Research ☒ No Change Since Previous Submission ☐ Change

D. Multiple PD/PI Leadership Plan ☒ No Change Since Previous Submission ☐ Change

E. Human Embryonic Stem Cell Line(s) Used ☒ No Change Since Previous Submission ☐ Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

None

Progress Report Summary

A. Specific Aims

Under the provisions of the 20 years of DSHEA, the FDA has primary responsibility for ensuring that appropriate regulatory actions are taken against marketed products that present significant health risks or bear false or misleading label claims. Foundational to the evaluation of such risks and claims is an adequate scientific base for decision-making. For botanical dietary supplements (BDS), development of such a science base is especially problematic because of several unique features, including the complexity of the constituents, variability of sourcing, lack of availability of reference materials, lack of good manufacturing controls and rapidly expanding use in the marketplace. In 2010 the FDA fully implemented the current good manufacturing practices (cGMP) for the botanical dietary supplement industry placing the onus of "botanical identity and authenticity" on the manufacturers of botanical dietary supplements. However, these cGMP's have in many ways increased the complexity as to what constitutes a "scientifically valid method" for the determination of the identity and authenticity of botanical materials. To address these complex issues surrounding botanical identity, authenticity, safety and toxicity and more importantly, in continuation of our cooperative agreement with the FDA, the NCNPR has established the following specific aims to facilitate the research needs of the FDA:

1. Assist in the identification and development of a list of BDS and botanical ingredients based on safety concerns, trends, and knowledge of botanicals being marketed in the U.S., to prioritize for further research.
2. Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to FDA, for the assessment of their adulteration, safety and toxicity.
3. Exchange technical and scientific information, analytical methods, and reference material with FDA scientists and other stakeholders.
4. Collaborate with FDA scientists in research areas of mutual interest.
5. Coordinate scientific workshops and conferences on BDS-related topics of public health relevance to address high priority science and awareness of emerging problems associated with botanicals to the public.

B. Studies and Results:

Aim 1: Assist in the identification and development of a list of BDS and botanical ingredients based on safety concerns, trends, and knowledge of botanicals being marketed in the U.S., to prioritize for further research.

Since the inception of the cooperative agreement between the NCNPR and the FDA in 2001, there have been numerous occasions for interactions between the two organizations. These have included (yearly) formal site visits by the FDA program officers, interactions during the annual International Conference on the Science of Botanicals (ICSB) and several conference calls and email exchanges. In addition to these interactions, over the past year the NCNPR has hosted five training sessions with the Office of Regulatory Affairs (ORA) to provide hands-on training to FDA inspectors for cGMP compliance issues associated with BDS's (FD340). These training sessions have provided an opportunity for the programs project officer (Dr. Daniel Fabricant) and his colleagues to visit the NCNPR and stay abreast of the Center's ongoing developments. As always, researchers at the NCNPR are receptive to areas of study that are deemed necessary by the FDA for this project.

Aim 2: Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to FDA, for the assessment of their adulteration, safety and toxicity.

The availability of authenticated reference materials for botanicals is an essential first step in the development of analytical methodologies for the identification and quantitative analysis of botanical product(s). The NCNPR has been very successful in placing formal agreements with several international academic and governmental

institutions including Pakistan, India (FRLHT), South Africa, Central/South America, China with the development of the Sino-US TCM Research Center and the established Center for Research in Indian Systems of Medicine (CRISM - www.CRISM.net) with the departments of AYUSH and CSIR in India. The NCNPR has also established a Memorandum of Understanding (MOU) with the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Brazil and the Natural Products Utilization Research Unit (NPURU), USDA located in Oxford Mississippi. Recently the NCNPR has cultivated a productive relationship with the Chinese Pharmacopeia and Chinese FDA in order to obtain relevant authenticated Traditional Chinese Medicines (TCM's) and pertinent purified constituents for authentication purposes. With this mechanism, NCNPR has acquired more than 150 new constituents over the past year and the collaboration would facilitate NCNPR to aid in populating a botanical information portal for CFSAN/FDA and expanding the in-house repository. In 2013, as a part of collaboration, more than 125 samples of authenticated tea tree oils were obtained from Southern Cross University, Australia to assess the safety, development of authentication techniques and to provide samples for possible allergen testing for CFSAN's cosmetic program. Lastly, NCNPR established an agreement with Tshwane University of Technology, Pretoria, South Africa to exchange the traditional practices based on botanicals endemic to South Africa such as *Sutherlandia frutescens*, *Hoodia gordonii* and other related plant materials of interest to the FDA.

From these and other collaborative relationships, the NCNPR has been able to acquire over 9500 plant samples and herbal extracts, representing approximately 5000 species over the duration of this project. There is a continuous effort in acquiring commercial samples as well for authentication purposes in addition to assuring that the developed identity testing techniques for this project are applicable for finished products in various formularies. Taking all these sample types into account, there are over 16,000 samples within the NCNPR-FDA repository. All the plants and samples collected for this program have been assigned an inventory number and are cataloged in the NCNPR repository and voucher specimens of authentic samples are also maintained at the Thomas M. Pullen Herbarium in the Department of Biology at the University of Mississippi.

In addition to the repository, the NCNPR has a newly renovated Medicinal Plant Garden that maintains more than 300 species for selected growing (field, greenhouse and shade houses). The new facilities consist of two main buildings (4,362 sf. and 4,290 sf.) and four additional support buildings and structures sitting on approximately 5.25 acres of land. The new facility was dedicated as the Maynard W. Quimby Medicinal Plant Garden on April the 15th 2013 as a part of the 12th ICSB. The Medicinal Plant Garden has developed significant collaborations with seed banks from 40 institutes around the world in order to exchange medicinally important germplasms. This seed bank effort has yielded a collection of over 1530 species to date. In addition, the garden personnel are preparing herbarium voucher specimens from the living collection and have started collecting samples for a DNA tissue bank. Over the course of this program, the garden provided 320 authentic reference samples from the living collection for the Center's research efforts. The Medicinal Plant Garden is also registered under the Botanic Gardens Conservation International and is a member of the American Association of Botanical Gardens and Arboreta and International Plant Exchange Network (IPEN). Overall, this new facility provides not only an invaluable resource for propagating and sourcing botanicals of interest but also provides a training facility for FDA/ORA courses on identification of botanical of interest.

Isolation of reference compounds: The isolation of standard compounds is required to develop analytical methods, quantify individual compounds in extracts and to establish analytical fingerprints in support of authenticity, quality, safety, and toxicity studies. Scientists at the NCNPR have isolated a number of compounds from species such as *Mitragyna speciosa*,¹ *Dioscorea villosa*,² *Dioscorea cayenensis*,³ *Dioscorea nipponica*,⁴ *Matricaria recutita* L., *Anthemis nobilis*, and *Lepidium meyenii*⁵ (*Maca*). It is through these continued efforts that the NCNPR scientist have isolated several novel compounds as well as known analogs for analytical finger printing development for authentication purposes as well as safety analysis.

Synthesis and procurement of compounds of interest: Under certain situations, synthesis of reference compounds is also undertaken at NCNPR wherein isolation of marker compounds was laborious and time consuming. Several sympathomimetic amines such as *N,N*-diethylphenethylamine, *N,N*-dimethylphenethylamine, phenethylamine, cocularine were synthesized from commercially available raw

materials on a bulk scale. In addition to large scale synthesis, several single enantiomers were synthesized for the development of analytical methods to understand the origin (synthetic/natural) of compounds of interest.

Analytical method development and metabolomic profiling: In the past year we have focused on developing several analytical methods for the determination of authenticity BDS's of interest. These studies include the development of UPLC (UV, ELS and MS), Supercritical Fluid Chromatography (SFC), standard HPLC/HPTLC analytical methods as well as using proton NMR for metabolomic profiling for common botanicals including *Matricaria recutita* L., (German chamomile) *Anthemis nobilis*, (Roman chamomile), *Chrysanthemum morifolium* (Chinese chamomile); determination of coumarin in Cinnamon species⁶ (*Cinnamomum verum*, *C. cassia*, *C. loureiroi*, and *C. burmannii*); *Terminalia* species⁷⁻⁹; *Dioscorea* species (*Dioscorea villosa* L., *D. cayennensis* Lam., *D. rotundata*, *D. opposita*, *D. caucasica*, *D. bulbifera*, *D. deltoidea*, *D. quaternata*); *Mitragyna speciosa*¹⁰; ¹¹ Korth; *Dendrobium nobile*; pyrrolizidine alkaloids from *Asteraceae*, *Boraginaceae*, *Fabaceae*; multifarious skin whitening agents; estimation of glucose¹² and steiviol glycosides; *Pelargonium graveolens*; *Serenoa repens*; and *Prunus africana*. Most importantly, the Center aided in developing an analytical approach establishing the absence of dimethylamylamine (DMAA) in authenticated *Pelargonium graveolens*. This newly developed method provided the FDA with the information required to challenge the marketing of DMAA products for lack of safety evidence on April 27, 2012. The results of this study were used to support FDA's position that DMMA found in dietary supplements sold in the U.S. (at >1 mg/g) must be synthetic. This finding led to the issuance by the agency of 10 warning letters to manufacturers and distributors of dietary supplements containing DMMA for which evidence of the safety of the product had not been submitted to FDA. The agency furthered warned the companies that synthetically-produced DMMA is not a "dietary ingredient" and, therefore, is not eligible to be used as an active ingredient in a dietary supplement.

Aim 3: Exchange technical and scientific information, analytical methods, and reference material with FDA scientists and other stakeholders.

Dr. Ikhlas Khan, Scientific Director for the project, has been in touch regularly with Dr. Daniel Fabricant (CFSAN, Director, Division of Dietary Supplement Programs), Dr. Elizabeth Calvey (CFSAN liaison), Dr. Diego Rua (CFSAN) and Dr. Robert L. Sprando, (CFSAN/OARSA, Director, Division of Toxicology) to exchange information on developed methods, reference materials availability, safety evaluations and project direction. These meetings and calls are designed to provide these individuals with an update of the NCNPR's overall program and to gain insight as to how this Center can diligently address the needs of the FDA.

Additionally, the NCNPR FDA-COE has also continued to assist the FDA's Office of Regulatory Affairs (ORA) in training inspectors for cGMP compliance for BDS's (FD340). Researchers at the NCNPR provided four one-day workshops on botanical dietary supplement authentication techniques to 135 trainees and FDA officials on May 20th, 2013, June, 19th, 2013, April 9th, 2014, May 7th, 2014 and scheduled two one-day workshops on, June 25th, 2014 and September 10th, 2014. The main training course is held in Memphis, Tennessee so that the trainees can attend a one-day excursion to the NCNPR for a combination of lectures and laboratory courses and training sessions to see what authentication techniques can be implemented for BDS's. The course covered current techniques utilized to identify botanical materials (Microscopy, Taxonomy, Macroscopy, TLC, HPLC, UPLC, GC, CE, etc.) and was presented by Dr. Khan and colleagues at the NCNPR and included two one and one half hour lab courses on Analytical Methods, Botanical Authentication, and Nomenclature. It is anticipated that this training program will continue for the foreseeable future and will provide the FDA cGMP botanical dietary supplement inspectors a valuable tool that will assist in their ability to carry out their duties.

Researchers at the NCNPR have also provided their expertise in other training offered by the FDA/ORA/DHRD. One such course was an advanced level course for analysts who are performing regulatory sample analysis using mass spectrometry techniques for identification and authentication (LB 403). Specifically, Dr. Yan-Hong Wang provided a lecture to several FDA trainees on August 28th– 29th 2013 covering the topic of how mass spectroscopy can be utilized for the authentication of botanicals. As an extension of this joint training with JIFSAN, the NCNPR sent Dr. Suman Chandra to provide four presentations at a GAP & GMP workshop aimed at supply chain management for spices and botanical ingredients for the Indian spice board (September 17th–21st, 2012, Kochi, India). Dr. Chandra covered topics such as harvesting

considerations, transportation/processing, cleaning and sanitation techniques. This workshop was then expanded into a multi-day multi-site visit for several members of the Indian spice board to provide these individuals with onsite training and lectures to provide advanced GAP and GMP techniques. This workshop, entitled "Food Safety and Supply Chain Management for Spices and Botanical Ingredients" was hosted by JIFSAN from March 25th - April 2nd then the NCNPR from April 3rd - 5th 2013. Dr. Ikhlas Khan attended the Spices Board India and All India Spice Exporters Forum, organized the World Spice Congress (WSC) in Cochin on February 16th-19th 2014. The highlight of the Congress was the Theme 'Sustainability and Food Safety' which is very relevant and crucial to the current scenario in the food sector. The business sessions planned by the Congress was led by globally renowned industry experts and addressed the topics on sustainable agriculture programs and practices based on real time experiences, infrastructural development, harmonization and simplification of standards etc. He also used this time to discuss the current collaboration between JIFSAN and the Indian Spice Board. In addition to above scientific activity, Dr. Khan and Dr. Troy Smillie participated in the 3rd Annual FDA Foods and Veterinary Medicine Science and Research Conference held in College Park, MD on August 27th -28th, 2013.

Lastly, the research effort initiated by the establishment of this Center has provided several opportunities to leverage the existing cooperative agreement so as to expand the impact and overall benefit of the existing program. This includes a recently funded NCCAM/ODS Botanical Research Center grant to explore Botanical Estrogens: Mechanisms, Dose and Target Tissues (PD: Helferich, William G., University of Illinois/PL: Khan, Ikhlas, A., Award number 5 P50 AT006268-02). Under this grant the NCNPR is providing significant quantities and populations of authenticated samples of Licorice - *Glycyrrhiza glabra* Linné var *glabra*, and Wild Yam - *Dioscorea villosa* L., for the established BRC.¹³ In addition to obtaining the outlined authenticated species for this program, we have established a substantial growing and collection program for closely related species for authentication and comparative purposes. Additionally, the NCNPR has obtained funding from the USDA/ARS for a grant entitled Discovery and Development of Natural Product-Based Insect Management Compounds for Medicinal, Veterinary and Urban Concern, award number 58-6402-1-612. The purpose of this grant is to discover and develop new, safer and more effective insect management products derived from natural phytochemical sources.

Aim 4: Collaborate with FDA scientists in research areas of mutual interest.

Over the past year the NCNPR has provided the FDA with scientific information for botanicals of concern as well as investigated several plants that have purported adverse events. Most notably we have undertaken an investigation of "chamomile" which has had reports of contact dermatitis and potential severe cases of allergic reactions in cosmetic formulations. There are two main species of "chamomile" utilized for commerce within the United States German chamomile, *Matricaria recutita* L.¹⁴ and Roman chamomile, *Anthemis nobilis*. Typically the flowering tops are used for most cosmetic formulations and these are either added as powdered material or an extract (ethanolic, supercritical or steam distilled). Working closely with scientists in the FDA/CFSAN office of cosmetics and colors, we initiated an extraction and bioassay guided fractionation of both German and Roman chamomile utilizing an LLNA screening assay for lead identification. Initial results are indicated that there is a potential sensitizer(s) within *Matricaria recutita* L. that could be causing the purported adverse events. Simultaneously, scientists at NCNPR developed two complementary *in chemico* (non-biological, non-animal) methods for identification and classification of chemical compounds as potential skin sensitizers, using either Nuclear Magnetic Resonance (NMR) spectroscopy or High Throughput Spectrophotometric methods. Further investigation including isolation, purification and *in chemico* evaluations indicated that the photo-oxidative metabolite of tonghaosu as a potential sensitizer in *Matricaria recutita* L. Scale-up, *in vivo* confirmation with LLNA screening assay have been undertaken and are still in progress.

A second project undertaken for CFSAN's office of cosmetics and colors looked at products that include an essential oil known as "Tea tree oil", which is obtained from several species of *Melaleuca* plants. One general research objective for this project is to seek further knowledge on compositional differences between commonly used species of *Melaleuca* plants (*M. alternifolia*, *M. linariifolia*, *M. viridiflora*, *M. leucadendron*, *M. dissitiflora*, etc.) in order to explore ways to address safety concerns for these species. The main safety concern about essential oils from these plant species is the potential for adverse effects on the skin, in

particular sensitization or allergic contact dermatitis (ACD). It is not fully understood which of the tea tree oil constituents is responsible for ACD. Therefore, the potential ACD active(s) in tea tree oil are currently being identified utilizing recently developed in-house NMR and HTS screening methods. Concurrently, we have developed an analytical GC/MS method to differentiate between the various species of tea tree oil that also identifies the major constituents. This newly developed method can be used to help identify potential ACD's within products.

NCNPR established two in-house preliminary *in-vivo* safety evaluations for botanicals of interest. Priority evaluation was given to botanicals that have displayed an inherent risk based on literature information or reported adverse events. Initial screens focused on two areas of concern. The first mouse model measured botanicals for their potential to induce positive reinforcement or cause aversive properties using a conditioned place preference (CPP) paradigm procedure that is commonly used to evaluate drugs for their "addictive" behavior. Over the past year the Center used this model to evaluate *Salvia divinorum*, *Mitragyna speciosa*, *Sceletium tortuosum* and fractions and pure compounds isolated from these species. Scientists at NCNPR employed the place preference/aversion paradigm to characterize the psychoactive properties of *Salvia divinorum* ext. (10, 30, 100 mg/kg), salvinin A (0.1, 0.3, 1.0 mg/kg), *Mitragyna speciosa* MeOH ext. (50, 100, 300 mg/kg), *Mitragyna speciosa* alkaloid-enriched fraction (12.5, 25, 75 mg/kg) and mitragynine (5, 10, 30 mg/kg) in rats. For *S. divinorum* the preliminary results indicated that this particular botanical did not induce abusive potential. For *M. speciosa* the results indicated that the major pharmacologically active constituent, mitragynine, has an abuse potential.¹⁵ Moreover, we have undertaken to study ADME properties of these compounds and their effect on the major efflux transporter P-glycoprotein, using *in vitro* methods. The stability of major alkaloids, mitragynine, 7-hydroxymitragynine and mitraphylline were subjected to Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF).¹⁶ All three compounds exhibited high plasma protein binding (> 90%) determined by equilibrium dialysis. Mitragynine and 7-hydroxymitragynine inhibited P-glycoprotein with EC₅₀ values of 18.2 ± 3.6 µM and 32.4 ± 1.9 µM, respectively, determined by the calcein-AM fluorescent assay, while no inhibition was seen with mitraphylline. These data suggest the possibility of a drug interaction if mitragynine and 7-hydroxymitragynine are co-administered with drugs that are P-glycoprotein substrates.

As a part of phytochemical investigation, we have isolated several known and unknown alkaloids from *S. tortuosum*. Noticeably, these marker components will assist us in authentication, identification and development of analytical methods for *S. tortuosum* and its principle alkaloid components. The majority of these findings have not yet been published; however, they will be reported shortly. Along with ADME properties and intravenous plasma pharmacokinetics, the behavioral studies associated with effects of *Sceletium tortuosum* in rats are still in progress and the results will be reported in due course.

The second *in-vivo* assay was developed to evaluate the potential hepatotoxicity's associated for botanical as measured in a mouse model where the hepatotoxicity is manifested by elevation of aminotransferases and histopathological features of acute hepatocellular necrosis and/or biliary injury. Initial *in-vivo* hepatotoxicity evaluations of EGCG,¹⁷⁻²⁰ a major component in green tea products, at high doses can lead to mild liver injury and under febrile conditions can cause severe liver injury. Currently, we are testing hepatotoxicity potential of OxyElite Pro and Black Cohosh in mice and results will be reported in due course. Both *in-vivo* models will continue to provide significant insight into the safety profile for botanicals that are of concern to public health.

Lastly, NCNPR has provided the FDA (CFSAN/OARSA) with scientific information for botanicals of concern as well as investigated several plants that are purported to have hepatotoxicity. Working closely with Dr. Sprando and his colleagues' at OARSA, we identified several sympathomimetic compounds and extracts reported to have hepatotoxic potential. Based on their usage in BDS, five whole methanolic extracts of *Astragalus membranaceus*, *Rauvolfia serpentina*, *Calea zacatechichi*, *Psoralea corylifolia*, *Adhatoda zeylanica*, *Kigelia Africana* were provided to OARSA to estimate the potential toxicity. In addition to these extracts, nine pure compounds were also provided to OARSA. Of nine pure compounds, based on practicality and other factors, four compounds, *N,N*-diethylphenethylamine, *N,N*-dimethylphenethylamine, phenethylamine, coclaurine were further selected for animal studies. At this point, 5.0 Kg of coclaurine was provided to OARSA and the findings will be reported in due course.

Aim 5: Coordinate scientific workshops and conferences on BDS topics of public health relevance to address high priority science and research needs.

The Center hosted the 13th Oxford International Conference on the Science of Botanicals (ICSB) that was held on April 15th – 17th, 2014, at The University of Mississippi to commemorate the 20 years of DSHEA. In addition to regulatory aspects with perspectives from government, manufacturers and trade associations; post market surveillance, risk and safety assessment, quality control and adverse event reporting (AER) for botanical dietary supplements (BDS) and natural products were discussed at 13th ICSB. This conference was co-sponsored by the Shanghai Institute of Materia Medica/CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant and Natural Products Research (GA); the Korean Society of Pharmacognosy and the Vietnam Academy of Science and Technology (VAST) the St Petersburg Institute of Pharmacy; the Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD) and the International Council on Medicinal and Aromatic Plants for Human Welfare (ICMAP) as such included representative delegations of scientists from various organizations in China, India and Europe. Due to the focused scheduling of the 13th ICSB, contributed presentations or poster submissions were not accepted. These types of presentations are reserved for the upcoming 2014 ASP/14th ICSB (<http://asp2014.org/>) which will be hosted in August 2nd - 6th 2014 Oxford, MS. This conference will cover the topics of TCM, Ayurveda, cultivation, collection, post-harvest, processing practices, identification, authentication and quality/safety assessment of botanicals including current clinical evaluations, regulatory aspects and the impact on the global dietary supplement industry. Again the proceedings from these conferences are expected to be published in *Planta Medica*.

C. Significance:

Plant collection, authentication, voucher specimens, isolation, synthesis of reference compounds and method development provide the basic tools for assessing the safety and quality of botanical products. Significant progress has been made by the NCNPR in each of these areas for several botanicals of interest over the past year. The acquired information, physical samples (plants, extracts, etc.) and phytochemical standards are freely available to researchers at the FDA for the evaluation purposes. Publications from this project have significantly contributed to the available methodological literature for the FDA and NCNPR (see references).

D. Plans:

To address the needs of the FDA on safety of BDS, Dr. Ikhlas Khan, Scientific Director for the project, would be in touch with Director, Division of Dietary Supplement Programs and liaison at CFSAN. The center will continue to exchange information on developed methods, reference materials availability, safety evaluations and project direction with CFSAN. Similar to DMAA, continual research effort will also focus on presence/absence of several alkaloids of concern for their potential safety concern due to their abuse potential, undesired adrenergic, dopaminergic receptor activities. For example, hydrastine, berberine, berberastine, hydrastinine, tetrahydroberberastine, canadine, and canalidine from *Hydrastis Canadensis*,²¹ yohimbine²² and related alkaloids from *Pausinystalia yohimbe* which is widely used as a supplement for bodybuilding and to enhance male sexual performance; Aegeline, several tetrahydroisoquinoline compounds such as boldine, reticuline and related compounds from *Aegle marmelos*.

Furthering the collaborative research effort we have established with CFSAN's office of cosmetics and colors, additional effort will continue to look at several potential areas of concern. The first being the continued exploration of products that contain "tea tree" essential oil(s) which can be derived from several species of *Melaleuca* plants. In addition to compositional differences between commonly used species of *Melaleuca* plants (*M. alternifolia*, *M. linariifolia*, *M. viridiflora*, *M. leucadendron*, etc.); the main research objective for this project will be to seek further knowledge on the sensitization potential of these essential oils using recently developed in-house in chemico methods and compare, validate the resulting data with KeratinoSens, direct peptide reactivity assay (DPRA). At the same time, we will also explore the stability, aging, isolation and identification of possible reactive intermediate(s) in these oils using the recently developed GC-MS analytical method.

Continual research effort will also focus on the two recently developed in-house *in-vivo* screen evaluating botanicals for their potential to induce positive reinforcement or cause aversive properties using the developed CPP paradigm procedure that is commonly used to evaluate drugs for "addictive" behavior and the second assay which evaluates potential hepatotoxicity associated for certain botanicals. Priority evaluation will be given to botanicals that have displayed an inherent risk based on literature information or reported adverse events. Both models will provide significant insight as to the safety profile for botanicals that are of concern to the public health.

The PI and Scientists at NCNPR will continue to work with OARSA by exchanging the scientific information on botanicals of concern with hepatotoxicity potential. For animal studies purpose, on bulk scale, three other compounds, *N,N*-diethylphenethylamine, *N,N*-dimethylphenethylamine, phenethylamines would be provided to OARSA. By working closely with OARSA, the Center will continue to provide significant insight into the authentication, validation, analytical and safety profile for BDS that are of hepatotoxic concern to public health.

To collect the information from labels which can help in determining the quantity of any given dietary ingredient, a label database will be developed. This database will assist in exposure calculations and report generation for general ingredient risk assessment. This information will be used internally as well as for public good.

Lastly, expansion of the existing repository of reference plant samples for the genera noted throughout this report will be a continuing priority for this project. New botanicals will be selected based on input received from the FDA program officer, collaborators and liaison for further studies and to evaluate their safety and quality. A fifteenth Oxford International Conference on the Science of Botanicals (ICSB) is proposed to be held on April 13th – 16th, 2015, Oxford, MS. The conference will be co-sponsored by the Shanghai Institute of Materia Medica/CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the Ministry of Indigenous Medicine, Sri Lanka; the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant and Natural Products Research (GA); the Korean Society of Pharmacognosy and the Vietnam Academy of Science and Technology (VAST) the St Petersburg Institute of Pharmacy; the Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD) and the International Council on Medicinal and Aromatic Plants for Human Welfare (ICMAP). This conference will cover the topics of TCM, Ayurveda, cultivation, collection, post-harvest, processing practices, identification, authentication and quality/safety assessment of botanicals including current clinical evaluations, regulatory aspects and the impact on the global dietary supplement industry. Again the proceedings from this conference are expected to be published in *Planta Medica*.

References

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ALL PERSONNEL REPORTGRANT NUMBER
U01FD004246

Place this form at the end of the signed original copy of the application. Do not duplicate.

Always list the PD/PI(s). In addition, list all other personnel who participated in the project during the current budget period for at least one person month or more, regardless of the source of compensation (a person month equals approximately 160 hours or 8.3% of annualized effort). Use the following abbreviated categories for describing Role on Project:

- PD/PI*
- Co-Investigator
- Faculty
- Postdoctoral (scholar, fellow, or other postdoctoral position)*
- Technician
- Staff Scientist (doctoral level)
- Statistician
- Graduate Student (research assistant)
- Non-student Research Assistant
- Undergraduate Student
- High School Student
- Consultant
- Other (please specify)

If personnel are supported by a Reentry or Diversity Supplement please indicate such after the Role on Project, using the following abbreviations: RS - Reentry Supplement; DS - Diversity Supplement.

*Commons ID required for any personnel holding this Role on Project and for all individuals supported by a Reentry or Diversity Supplement. The Commons ID will be required in the future for all individuals with a graduate student, or undergraduate role. The Commons ID is strongly encouraged, but not required, for all other Project Personnel.

Use Cal (calendar), Acad, or Summer to enter months devoted to project.

Commons ID*	Name	Degree(s)	SSN (last 4 digits)	Role on Project	DoB (MM /YY)	Cal	Acad	Summer
IKHLAS	Ikhlas Khan	Ph.D.	(b) (6)	PI	(b) (6)	5.4		
LARRYWA LKER2004	Larry Walker	Ph.D.		Co-PI		1.2		
SKHAN	Shabana Khan	Ph.D.		Staff Scientist		2.76		
BAVULA	Bharathi Avula	Ph.D.		Staff Scientist		8.4		
YAN HONG	Yan Hong Wang	Ph.D.		Staff Scientist		12		
	Natasha Techen	Ph.D.		Staff Scientist		12		
ALI	Zulfiqar Ali	Ph.D.		Staff Scientist		3		
CHITTIBOY INA	Amar Chittiboyina	Ph.D.		Staff Scientist		6		
	Gouyi Ma	Ph.D.		Staff Scientist		12		
	Ahmad Osman	Ph.D.		Staff Scientist		12		
JPZHAO	Jianping Zhao	Ph.D.		Staff Scientist		6.36		
VRAMAN	Vijayasankar Raman	Ph.D.		Postdoc		12		
	Mei Wang	Ph.D.		Postdoc		8.4		

ALL PERSONNEL REPORTGRANT NUMBER
U01FD004246

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Always list the PD/PI(s). In addition, list all other personnel who participated in the project during the current budget period for at least one person month or more, regardless of the source of compensation (a person month equals approximately 160 hours or 8.3% of annualized effort). Use the following abbreviated categories for describing Role on Project:

- PD/PI*
- Co-Investigator
- Faculty
- Postdoctoral (scholar, fellow, or other postdoctoral position)*
- Technician
- Staff Scientist (doctoral level)
- Statistician
- Graduate Student (research assistant)
- Non-student Research Assistant
- Undergraduate Student
- High School Student
- Consultant
- Other (please specify)

If personnel are supported by a Reentry or Diversity Supplement please indicate such after the Role on Project, using the following abbreviations: RS - Reentry Supplement; DS - Diversity Supplement.

*Commons ID required for any personnel holding this Role on Project and for all individuals supported by a Reentry or Diversity Supplement. The Commons ID will be required in the future for all individuals with a graduate student, or undergraduate role. The Commons ID is strongly encouraged, but not required, for all other Project Personnel.

Use Cal (calendar), Acad, or Summer to enter months devoted to project.

Commons ID*	Name	Degree(s)	SSN (last 4 digits)	Role on Project	DoB (MM /YY)	Cal	Acad	Summer
	Christina Avonto	Ph.D.	(b) (6)	Postdoc	(b) (6)	12		
	Amira Wanas	Ph.D.		Postdoc		6		
	Gray Dale	MA		Rsch Asst		3		
	Zhihao Zhang	Ph.D.		Postdoc		12		
	Pradip Lasonkar	Ph.D.		Postdoc		12		
	Iffat Parveen	Ph.D.		Postdoc		12		
	Vamshikrishna Manda	Ph.D.		Postdoc		12		
	Min Hye Yang	Ph.D.		Postdoc		12		
	Satyanarayanaraju Sagi	Ph.D.		Postdoc		12		
	Helaina Craig	BA		Rsch Asst		12		
	Lal Jayaratna	MSc		Rsch Asst		12		
	Steven Hopper	BFA		Technician		12		
	Jennifer Taylor			Rsch Asst		12		

Department of Health and Human Services
Public Health Services

Review Group

Type

Activity

Grant Number

1U01FD004246

Grant Progress Report

Total Project Period

From: 09/15/2011

Through: 08/31/2016

Requested Budget Period

From: 09/1/2015

Through: 08/31/2016

1. TITLE OF PROJECT

Science Based Authentication of Dietary Supplements

2a. PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR

(Name and address, street, city, state, zip code)

Khan, Ikhlas A.
120 Faser Hall/NCNPR
School of Pharmacy
University of Mississippi
University, MS 38677

2b. E-MAIL ADDRESS

ikhan@olemiss.edu

2c. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT

National Center for Natural Products Research

2d. MAJOR SUBDIVISION

School of Pharmacy

2e. Tel: 662-915-7821

Fax: 662-915-7989

3a. APPLICANT ORGANIZATION

(Name and address, street, city, state, zip code)

The University of Mississippi
Office of Research and Sponsored Programs
100 Barr Hall PO Box 907
University, MS 38677

3b. Tel: 662-915-7482

Fax: 662-915-7577

3c. DUNS: 067713560

4. ENTITY IDENTIFICATION NUMBER

1646001159A1

6. HUMAN SUBJECTS

☒ No☐ Yes6a. Research
Exempt☒ No ☐ Yes

If Exempt ("Yes" in

6a):

Exemption No.

If Not Exempt ("No" in

6a):

IRB approval date

5. NAME, TITLE AND ADDRESS OF ADMINISTRATIVE OFFICIAL

Robin Buchannon, Asst. V.C. for Research and
Sponsored Programs, Research and Sponsored
Programs, 100 Barr Hall, University MS 38677

Tel: 662-915-7482

Fax: 662-915-7577

E-MAIL: research@olemiss.edu

6b. Federal Wide Assurance No.

6c. NIH-Defined Phase III

Clinical Trial ☒ No ☐ Yes7. VERTEBRATE ANIMALS ☐ No ☒ Yes

7a. If "Yes," IACUC approval Date 06-20-12

7b. Animal Welfare Assurance No. A3356-01

10. PROJECT/PERFORMANCE SITE(S)

Organizational Name: University of Mississippi

DUNS: 067713560

8. COSTS REQUESTED FOR NEXT BUDGET PERIOD

8a. DIRECT \$1,774,091

8b. TOTAL \$2,500,000

Street 1: 120 Faser Hall/NCNPR

Street 2: School of Pharmacy

9. INVENTIONS AND PATENTS ☒ No ☐ YesIf "Yes," ☐ Previously Reported☐ Not Previously Reported

City: University

County: Lafayette

State: MS

Province:

Country: USA

Zip/Postal Code: 38677

Congressional Districts: MS-001

11. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Item 13)

Robin Buchannon, Assistant Vice Chancellor for Research and Sponsored Programs

TEL: 662-915-7482

FAX: 662-915-7577

E-MAIL: research@olemiss.edu

12. Corrections to Page 1 Face Page

13. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties

SIGNATURE OF OFFICIAL NAMED IN
11. (In ink)

Robin C. Buchannon

DATE

5-29-15

Program Director/Principal Investigator (Last, First, Middle): Khan, Ikhlas, A.

			Salary Req.	Fringe	Total
Personnel					
PI	Ikhlas Khan	50%	(b)	(6)	
Co-PI	Larry Walker	10%			
Sr. Research Scientist	Shabana Khan	25%			
Assoc. Res. Biologist	Jessica Carpenter	100%			
Res. Scientist	Bharthi Avula	80%			
Sr. Research Scientist	Yan Hong Wang	100%			
Res. Scientist	Natascha Tehen	100%			
Res. Scientist	Zulfiqar Ali	95%			
Res. Scientist	Amar Chittiboyina	60%			
Res. Scientist	Gouyi Ma	100%			
Res. Scientist	Ahmad Osman	100%			
Assoc. Res. Scientist	Jianping Zhou	53%			
Res. Scientist	Vijayasankar Raman	100%			
Post Doc	Mei Wang	95%			
Post Doc	Cristina Avonto	100%			
Post Doc	Zhihao Zhang	100%			
Post Doc	Pradeep Lasonkar	100%			
Post Doc	Satyanarayanaraju Sagi	100%			
Post Doc	Ji-Yeong Bae	100%			
Post Doc	Iffat Parveen	100%			
R&D Botanist	Lal Jayaratna	50%			
Web Developer	Steven Hopper	100%			
Program Coordinator	Jennifer Taylor	100%			
Post Doc	Saqlain Haider	100%			
Post Doc	Sanaz Salehi	100%			
Project Coordinator	Gray Dale	10%			
Hourly Wages		100%			
Graduate Students (4)		100%			
Total Salaries and FB					\$1,386,527
equipment					\$70,000
supplies					\$153,268
travel					\$40,000
contractual services					\$70,000
MOBOT					\$54,296
Subtotal					\$1,774,090.55
F&A 44%					\$725,910
Total Request					\$2,500,000

Program Director/Principal Investigator (Last, First, Middle): Khan, Ikhlas, A.

BUDGET JUSTIFICATION

GRANT NUMBER
1U01FD004246

Provide a detailed budget justification for those line items and amounts that represent a significant change from that previously recommended. Use continuation pages if necessary.

A. PERSONNEL: \$1,386,527

Faculty and Professional Staff

PI, Dr. Ikhlas A. Khan, Assistant Director and Research Professor, National Center for Natural Products Research, Principal Investigator will devote 50% of his time to this program. Dr. Khan will be the contact point with NCNPR for interactions with FDA scientists. Dr. Khan has extensive research project administrative experience, has served as PI of several projects. Dr. Khan is currently working as a program director of FDA program at the Center. He works directly with Dr. Walker on a daily basis for scientific direction of major portions of NCNPR research efforts.

Co-Investigator, Dr. Larry A. Walker, Director, National Center for Natural Products Research, Co-Principal Investigator will provide the time & effort necessary for the overall administrative direction of the program. Dr. Walker will organize and coordinate the Steering Committee operation, and work closely with FDA officials and University administration to ensure administrative compliance and performance. No costs will be incurred to the grant for Dr. Walker's support.

CURRENT BUDGET PERIOD

FROM
09/01/15

THROUGH
8/31/16

Explain any estimated unobligated balance (including prior year carryover) that is greater than 25% of the current year's total budget.
N/A

BUDGET JUSTIFICATION CONTINUATION

Faculty and Professional Staff Continued

Principal Research Scientist (Dr. Shabana I. Khan) - 25% effort. Dr. Khan will be responsible for direction of the efforts at UM to evaluate toxicological parameters for the natural products and botanical extracts. She will commit to the project. Dr. Khan's training is in biochemistry, and she has worked for many years in the screening development with natural products. She will supervise the efforts of the toxicology research associates.

Sr. Research Scientist, Synthetic Chemist (Dr. Amar Chittiboyina) – 60% effort. Dr. Chittiboyina will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards. Dr. Chittiboyina will be responsible for all scientific aspects of data management for the project. Dr. Chittiboyina will coordinate particularly with the botanists, geneticist, analytical and isolation chemistry investigators, as well as with FDA scientists involved in the project, to develop and modify the data management workflow.

Sr. Research Scientist, Analytical Chemist (Dr. Bharathi Avula) – 80% effort. Research Scientist, National Center for Natural Products Research will be responsible for analytical method development. Dr. Avula is a trained pharmacist and analytical chemist. She has developed expertise over the years in analysis of botanicals. She will also be supervising Dr.'s Yan Hong Wang, and Mei Wang in developing relevant analytical methods.

Senior Research Scientist, Analytical Chemist (Dr. Yan Hong Wang) – 100% effort. Dr. Wang will be responsible for analytical method development. Dr. Wang is a natural products chemist with analytical background. He has developed an extensive expertise over the years in the analysis of botanicals.

Research Scientist, Plant Genetics (Dr. Natscha Tehen) – 100% effort. Dr. Tehen will be responsible for plant genetics approaches applied to the authentication of botanical dietary supplements. She is experienced in all the molecular techniques needed to accomplish this task.

Research Scientist, Isolation Chemist (Dr. Zulfiqar Ali) – 95% effort. Dr. Ali will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Research Scientist, Biologist (Dr. Gouyi Ma) – 100% effort. Dr. Ma will be responsible for the development of in-vitro assays to assess the toxicological profile of botanicals

Research Scientist, Chemist – (Ahmad Osman) 100% effort. This individual will be responsible for isolating and identifying compounds of interest from various botanical sources. Additionally, this person will be instrumental in compiling scientific information with regard to identification techniques (analytical, NMR, etc) for the authentication of botanical dietary supplements for the purpose of populating a dynamic web portal to assist FDA researchers and other stakeholders.

Associate Research Scientist, Isolation Chemist (Dr. Jiaping Zhao) – 53% effort. Dr. Zhao's responsibility will be to prepare extracts of selected botanicals, develop NMR identification/authentication techniques, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Postdoctoral Research Associate, Botanist (Dr. Vijayasankar Raman) – 100% effort. Dr. Raman will be working on macro and microscopy of botanicals, needed for species authentication. Microscopy provides an understanding of anatomical features of a plant, which is instrumental in differentiating the species and also in determining potential adulterants.

Post Doctoral Research Associate, Analytical Chemist (Dr. Mei Wang) – 95% effort. Dr. Wang will develop analytical methods for the purpose of identifying botanical dietary supplement samples. Dr. Wang has several years of experience in developing analytical GC techniques for the purpose of analysis and quantitation.

Post Doctoral Research Associate, Chemist (Dr. Cristina Avonto) – 100% effort. Dr. Avonto will be responsible for isolating marker compounds and bioactive constituents from botanicals. Additionally Dr. Avonto will perform analytical profiling of botanicals using various GC techniques.

Post Doctoral Research Associate, Isolation Chemist (Zhihao Zhang) – 100% effort. Dr. Zhang will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Post Doctoral Research Associate, Isolation Chemist (Dr. Satyanarayanaraju Sagi) – 100% effort. Dr. Sagi will develop analytical methods for the purpose of identifying botanical dietary supplement samples. Dr. Sagi has several years of experience in developing analytical HPTLC/LC techniques for the purpose of analysis and quantitation.

Post Doctoral Research Associate, Synthetic Chemist (Dr. Pradeep Lasonkar) – 100% effort. Dr. Lasonkar will synthesize the various small molecule amines for behavioral studies. Along with synthesis he is also responsible for isolation and elucidation structures of potential marker or active compounds.

Research Associate (Jessica Carpenter) – 100% effort. To assess the abuse potential of select botanicals, Ms. Carpenter will perform the behavioral studies in various biological models such as conditioned place preference, force-swim test.

Post Doctoral Research Associate, Synthetic Chemist (Dr. Saqlain Haider) – 100% effort. Dr. Haider will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards. In addition to isolation work, he will be responsible for identification and synthesis of small molecules hepatotoxicity.

Post Doctoral Research Associate, Isolation Chemist (Ji-Yeong Bae) – 100% effort. Dr. Bae will prepare extracts of selected botanicals, isolate and elucidate structures of potential marker or active compounds, scale up isolation/purification to obtain reference standards.

Post Doctoral Research Associate, Microbiologist (Dr. Sanas Salehi) – 100% effort. Dr. Salehi will be responsible for identification and characterization of metabolites of select botanical ingredients with microbiota.

Post Doctoral Research Associate, Plant Genetics (Dr. Iffat Parveen) – 100% effort. Dr. Parveen will be responsible for plant genetics approaches applied to the authentication of botanical dietary supplements. She will assist and train under Dr. Tehen for the molecular techniques needed to accomplish the proposed work.

R&D Botanist (Lal Jayaratna) – 100% effort. This individual is responsible for maintaining the living collection of the centers medicinal plant garden. Additionally this individual prepares voucher specimens, maintains the programs seed bank and provides assistance/tours for training sessions and workshops.

R&D Data Analyst – (Steven Hopper) 100% effort. This individual will be responsible for the development of a botanical dietary supplement information portal for the dissemination of relevant scientific data and FDA Dietary Supplement cGMP information. In addition to this he will be aiding in the data labeling, collection/reporting efforts for this project.

Project Coordinator (Gray Dale) - 10% effort. Mr. Dale is responsible to the PIs. His work is necessary for management of reports, budgets and travel. Mr. Dale also provides vital logistical support for ICSB conference.

Technical and Administrative Staff

Program Coordinator (Jennifer Taylor) - 100% effort. Ms. Taylor is responsible to the PIs, and provides necessary follow-up with reports, publications, file documentation, sample tracking, etc. Ms. Taylor also provides vital logistical support for workshops, training sessions and conferences.

NOTE: The positions of Program Coordinator and Project Coordinator are normally not allowed as direct costs under the Uniform Guidance. However, we are requesting these direct charges be allowed due to the large scope of the project and the number of personnel to be managed and supported. The activities of the coordinators in managing the large staff are integral to the project.

Hourly Wages – Hourly wage support (\$13,790) will be utilized for temporary and student workers employed in support of the project. These may be utilized in support of administrative or research activities, ranging from filing and data entry to field/greenhouse or laboratory assistance.

Graduate Students (4) – The graduate students will receive training in natural products chemistry, analytical chemistry. These students will be enrolled in the Ph.D. program in the Department of Pharmacognosy, or one of the other academic Departments in the school of Pharmacy (\$89,904).

Fringe Benefits

Fringe benefits for faculty and staff are calculated at the University's standard rate of 32.75% of salary. Fringe benefits for graduate students are calculated at the University's standard rate of 8% of wages. For undergraduate students fringe benefits are calculated at the University's standard rate of 3% of wages.

B. CONSULTANT COSTS: None

C. EQUIPMENT: \$ 70,000

These funds are to support the capacity for analytical work that is required for the success of the program. These funds will be utilized to either purchase new complete systems or to upgrade other existing equipment such as HPLC, GC, NMR or MS.

D. SUPPLIES: \$ 153,268

Primary commodity expenditures for the project will be for:

HPLC columns \$22,947

NMR/MS supplies (tubes, gases, columns) \$13,700

Microscopic supplies (slides, stains, optics, mounting preparation) \$6,700

Chromatographic supplies (TLC plates, chromatographic media, solvents) \$38,921

Mol. Biology supplies \$30,000

Botanical collection/storage materials \$13,000

Garden/greenhouse tools/supplies \$12,000

Books, databases other reference materials \$12,000

Computer supplies \$4,000**

**These costs are for essential computer supplies which are devoted solely to the FDA project, and not for general use.

Sub Total: \$153,268

E. TRAVEL: \$40,000

Included in this category are:

Travel for FDA Dietary Supplement GMP training sessions.

Establishing collection arrangements and collaborations (National and International)

Travel to/from Washington or other COE's for meetings with FDA officials

Travel costs related to hosting workshop/conference (National and International)

F. CONTRACTUAL SERVICES: \$70,000

Estimated expenditures in this category include contractual/professional services for:

Collection & documentation of plant material \$9,000

scale-up extraction/isolation \$ 9,000

taxonomic verifications \$6,000

maintenance contracts/repairs for analytical equipment \$34,000

software/upgrades for analytical equip. \$8,000

shipping \$4,000

Sub-Total: \$70,000

Estimated expenses for hosting conference:

Printing/PR \$3,500

Speaker reimbursements (28 @ 1,500) \$42,000

Dinners/breaks \$11,000

Staffing \$8,000

Sub Total: \$64,500

G. SUBCONTRACT: \$54,296

A subcontract with Missouri Botanical Garden will be in place in the amount of \$54,296. Missouri Botanical Garden will be collecting the specimens for this project and will be providing expertise on identification of the specimens. Dr. Rainer Bussmann, the director and curator of the William L. Brown Center for Plant Genetic Resource (WLBC), will be the Missouri Botanical Garden representative working on the project.

H. INDIRECT CHARGES: \$ 725,910

Indirect costs are calculated in accordance with The University of Mississippi's rate agreement with DHHS, dated September 12, 2011. Indirect costs for research are calculated at 44.0% of Total Direct Costs less equipment, tuition remission, and the portion of each sub-grant or subcontract in excess of \$25,000.

Program Director/Principal Investigator (Last, First, Middle): Khan, Ikhlas A

PROGRESS REPORT SUMMARY	GRANT NUMBER 1U01FD004246	
	PERIOD COVERED BY THIS REPORT	
	FROM 09/1/2015	THROUGH 08/31/2016
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Khan, Ikhlas A		
APPLICANT ORGANIZATION The University of Mississippi		
TITLE OF PROJECT (Repeat title shown in Item 1 on first page) Science Based Authentication of Dietary Supplements		

A. Human Subjects (Complete Item 6 on the Face Page)

Involvement of Human Subjects ☒ No Change Since Previous Submission ☐ Change

B. Vertebrate Animals (Complete Item 7 on the Face Page)

Use of Vertebrate Animals ☒ No Change Since Previous Submission ☐ Change

C. Select Agent Research ☒ No Change Since Previous Submission ☐ Change

D. Multiple PD/PI Leadership Plan ☒ No Change Since Previous Submission ☐ Change

E. Human Embryonic Stem Cell Line(s) Used ☒ No Change Since Previous Submission ☐ Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

None

Progress Report Summary

A. Specific Aims

Under the provisions of the DSHEA for 21 years, the FDA has primary responsibility for ensuring that appropriate regulatory actions are taken against marketed products that present significant health risks; bear false or misleading label claims. Foundational to the evaluation of such risks and claims is an adequate scientific base for decision-making. For botanical dietary supplements (BDS), development of such a science base is especially problematic because of several unique features, including the complexity of the constituents, variability of sourcing, lack of reference materials, lack of good manufacturing controls and rapidly expanding use in the marketplace. In 2010 the FDA fully implemented the current good manufacturing practices (cGMP) for the botanical dietary supplement industry placing the onus of "botanical identity and authenticity" on the manufacturers of botanical dietary supplements. However, these cGMP's have in many ways increased the complexity of what constitutes a "scientifically valid method" for the determination of the identity and authenticity of botanical materials. To address these complex issues surrounding botanical identity, authenticity, safety and toxicity and more importantly, in continuation of our cooperative agreement with the FDA, the NCNPR has established the following specific aims to facilitate the research needs of the FDA:

1. Assist in the identification and development of a list of BDS and botanical ingredients taking account of safety concerns, trends, and knowledge of botanicals being marketed in the U.S., to prioritize for further research.
2. Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to the FDA, for the assessment of their fingerprinting, adulteration, safety and toxicity.
3. Exchange technical and scientific information, analytical methods, and reference material with FDA scientists and other stakeholders.
4. Collaborate with FDA scientists in research areas of mutual interest.
5. Coordinate scientific workshops and conferences on BDS-related topics of public health relevance to address high priority science and public awareness of emerging problems associated with botanicals.

B. Studies and Results:

Aim 1: Assist in the identification and development of a list of BDS and botanical ingredients taking account of safety concerns, trends, and knowledge of botanicals being marketed in the U.S., to prioritize for further research.

Since the inception of the cooperative agreement between the NCNPR and the FDA in 2001, there have been numerous occasions for interactions between the two organizations. These have included (yearly) formal site visits by the FDA program officers, interactions during the annual International Conference on the Science of Botanicals (ICSB), several monthly conference calls and numerous email exchanges. In addition to these interactions, over the past year the NCNPR has hosted five training sessions with the Office of Regulatory Affairs (ORA) to provide hands-on training of FDA inspectors for cGMP compliance issues associated with BDS's (FD340). Under this aim, a total of 153 inspectors were trained during 2014 calendar year. These training sessions have provided an opportunity for the Programs Project Officer (Dr. Cara Welch) and her colleagues to visit the NCNPR and stay abreast of the Center's ongoing developments. As always, researchers at the NCNPR are receptive to areas of study that are deemed necessary by the FDA for this cooperative agreement.

Aim 2: Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to the FDA, for the assessment of their fingerprinting adulteration, safety and toxicity.

The availability of authenticated reference materials for botanicals is an essential first step in the development of analytical methodologies for the identification and quantitative analysis of botanical product(s). The NCNPR

has been very successful in placing formal agreements with several international academic and governmental institutions including Pakistan, India (FRLHT), South Africa, Central/South America, China with the development of the Sino-US TCM Research Center and the established Center for Research in Indian Systems of Medicine (CRISM) with the departments of AYUSH and CSIR in India. The NCNPR has also established a Memorandum of Understanding (MOU) with the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Brazil and the Natural Products Utilization Research Unit (NPURU), USDA located in Oxford Mississippi. Recently the NCNPR has cultivated a productive relationship with the Chinese Pharmacopeia and Chinese FDA in order to obtain relevant authenticated Traditional Chinese Medicines (TCM's) and pertinent purified constituents for authentication purposes. With this mechanism, NCNPR has acquired more than 150 new constituents over the past year and the proposed collaboration would facilitate NCNPR to aid in populating a botanical information portal for CFSAN/FDA and expanding the in-house repository. In 2013, as a part of collaboration, more than 101 samples of authenticated tea tree oils were obtained from Southern Cross University, Australia to assess the safety, development of authentication techniques and to provide samples for possible allergen testing for CFSAN's cosmetic program. Lastly, NCNPR established an agreement with Tshwane University of Technology, Pretoria, South Africa to exchange the traditional practices based on botanicals endemic to South Africa such as *Sutherlandia frutescens*, *Hoodia gordonii* and other related plant materials of interest to the FDA.

From these and other collaborative relationships, the NCNPR has been able to acquire over 9500 plant samples and herbal extracts, representing approximately 5000 species over the duration of this project. There is a continuous effort to acquire authentic plant samples as well for standardization purposes in addition to assuring that the developed identity testing techniques for this project are applicable for finished products in various formularies. Taking all these sample types into account, there are over 17,130 samples within the NCNPR-FDA repository. All the plants and samples collected for this program have been assigned an inventory number and are cataloged in the NCNPR repository and voucher specimens of authentic samples are also maintained at the Thomas M. Pullen Herbarium in the Department of Biology at the University of Mississippi.

In addition to the repository, the NCNPR has a newly renovated Maynard W. Quimby Medicinal Plant Garden that maintains more than 300 species for selected growing in field, greenhouse and shade houses. The new facilities consist of two main buildings (4,362 and 4,290 sq ft) and four additional support buildings and structures sitting on 5.25 acres of land. The Medicinal Plant Garden has developed significant collaborations with seed banks from 40 institutes around the world in order to exchange medicinally important germplasms. This seed bank effort has yielded a collection of over 1730 species to date. In addition, so far, the garden personnel added 500 plant vouchers; are planning to add another 100 herbarium voucher specimens from the living collection and have started collecting samples for a DNA tissue bank. Over the course of this program, the garden provided 320 authentic reference samples from the living collection for the Center's research efforts. The Medicinal Plant Garden is also registered under the Botanic Gardens Conservation International and is a member of the American Association of Botanical Gardens and Arboreta and International Plant Exchange Network (IPEN). Overall, this garden provides not only an invaluable resource for propagating and sourcing botanicals of interest but also provides a training facility for FDA/ORA courses on identification of botanicals of interest.

Macroscopic techniques are routinely used in our labs to discriminate between the desired plant species or plant part, and morphologically similar, yet distinguishable, species that could occur as potential adulterants. Using macroscopy, more than 100 samples were authenticated based on their morphology. Microscopic techniques¹ are performed in our labs to assure authenticity or detect adulteration in ground plant samples where macroscopic characteristics are difficult to observe. For example, Buchu (*Agathosma betulina*) is a popular medicinal plant known for its beneficial properties such as diuretic, urinary tract antiseptic, stimulant and tonic. With microscopy, adulterations or contaminations with senna, grass and various other extraneous materials were observed in some of the buchu commercial samples. In addition to macro and microscopic studies, we also conduct DNA fingerprinting² studies to authenticate and validate the reference plant material. Several plant parts of *Glycyrrhiza* (licorice) and *Dioscorea* (wild yam) samples were analyzed by DNA fingerprinting, such as ITS sequences and/or LEAFY intron 2 genomic regions, to establish the species

variation. Such information is vital for establishing a single database containing information about authentic plant materials and their potential adulterants. Working closely with our Program Officer, Dr. Welch and DDSP, the scientists at NCNPR developed a botanical dietary supplement information portal for the dissemination of relevant scientific data and FDA Dietary Supplement cGMP information (CFR Dietary Supplement Labels Database).

Isolation of reference compounds: The isolation of standard compounds is required to develop analytical methods, quantify individual compounds in extracts and to establish analytical fingerprints in support of authenticity, quality, safety, and toxicity studies. Scientists at the NCNPR have isolated a number of compounds from Red Yeast Rice and species such as *Mitragyna speciosa*,³ *Melaleuca alternifolia*, *Rauwolfia vomitoria*, *Rauwolfia serpentina*, *Kigelia africana*, *Aframomum melegueta*, *Terminalia chebula*, *Chamaemelum nobile* (Roman chamomile), and *Pelargonium graveolens* L'Her⁴. It is through these continued efforts that the NCNPR scientist have isolated several novel compounds as well as known analogs for analytical finger printing development for authentication purposes as well as safety analysis.

Synthesis and procurement of compounds of interest: Under certain situations, synthesis of reference compounds is also undertaken at NCNPR when isolation of marker compounds was laborious and time consuming. Several compounds of interest, such as (±)-aegeline, demethylcoclaurine (higenamine), *N,N*-diethylphenethylamine, *N,N*-dimethylphenethylamine, were synthesized from commercially available raw materials on a bulk scale. In addition to large scale synthesis, several single enantiomers such as *S*-aegeline were synthesized for the development of analytical methods to understand the origin (synthetic/natural) of compounds of interest.

Analytical method development and botanical fingerprint profiling: In the past year we have focused on developing several analytical methods for the determination of authenticity BDS's of interest. These studies include the development of UPLC (UV, ELS and MS), Supercritical Fluid Chromatography (SFC), standard HPLC/HPTLC and data analysis in real time (DART)-QToF-MS analytical methods. For this reporting period, several botanicals including authentication of true cinnamon (*cinnamon verum*)⁵; characterization and screening of pyrrolizidine alkaloids and N-oxides from botanicals and dietary supplements⁶; profiling and quantification of monacolins and citrinin in dietary supplements containing red yeast rice⁷; quantitative determination of steroidal marker compounds for wild yam (*Dioscorea* spp.)^{8, 9}; differentiate chemo-types of chamomile¹⁰⁻¹²; detection of mitragynine and other (ox)indole alkaloids in *Mitragyna speciosa*^{13, 14}; ginkgolic acids content in *Ginkgo biloba* dietary supplements¹⁵; quality evaluation of terpin-4-ol type Australian tea tree oils and commercial products¹⁶; stereochemical tests for the identification and differentiation of *Pelargonium graveolens* L'Her. (Geraniaceae) essential oils⁴; quantitative determination of arbutin and other phenolic compounds in skin whitening products¹⁷ and quality and adulterant assessment of steviol glycosides sweeteners.¹⁸

1,3-Dimethylamylamine (DMAA) is a sympathomimetic compound currently incorporated into some dietary supplements. Significant controversy exists regarding the 'natural' origin of DMAA, as claimed by manufacturers of supplements. The NCNPR aided in developing an analytical approach¹⁹ establishing the absence of dimethylamylamine (DMAA) in authenticated *Pelargonium graveolens*. The results of this study were used to support FDA's position that DMAA found in dietary supplements sold in the U.S. (at >1 mg/g) must be synthetic. The agency warned the companies that synthetically-produced DMAA is not a "dietary ingredient" and, therefore, is not eligible to be used as an active ingredient in a dietary supplement. However, two other studies^{20, 21} reported the presence of DMAA in some samples of *P. graveolens* and pelargonium oil acquired by the investigators from China. Because of the appearance of two studies funded by USPlabs, LLC ("USPlabs"), purporting to have detected the presence of DMAA in *pelargonium* species or pelargonium oil, we initiated a multi-center study to determine if there is any validity to the studies.²² All four sites adopted similar extraction method as that reported by Li et al.²¹. Some of the geranium used in the study was collected by the same individual who collected samples for the Fleming et al., study²⁰ that found DMAA in samples from the same region. A total of 18 plant samples belonging to 6 different pelargonium species and 9 oils from different locations around the world were split among 4 different analytical laboratories for analysis (each lab received the same samples). None of the laboratories detected the presence of DMAA in any of the 27 samples at the

low detection levels obtainable with modern QToF instrumentation. Based on the best available detection techniques, the multi-center investigation demonstrates that DMAA is not a constituent of *P. graveolens*. These findings were published in Drug Testing & Analysis (First published online on 23rd October, 2014. DOI: 10.1002/dta.1726)

Aim 3: Exchange technical and scientific information, analytical methods, and reference material with FDA scientists and other stakeholders.

Dr. Ikhlas Khan, Scientific Director for the project, has been in touch regularly with Dr. Cara Welch (CFSAN, Director, Division of Dietary Supplement Programs), Dr. Elizabeth Calvey (CFSAN liaison), Dr. Patricia Hansen (CFSAN/OCC, Deputy Director) and Dr. Robert L. Sprando, (CFSAN/OARSA, Director, Division of Toxicology) to exchange information on developed methods, reference materials availability, safety evaluations and project direction. These meetings and calls are designed to provide these individuals with an update of the NCNPR's overall program and to gain insight as to how this Center can diligently address the needs of the FDA.

Additionally, the NCNPR FDA-COE has also continued to assist the FDA's Office of Regulatory Affairs (ORA) in training inspectors for cGMP compliance for BDS's (FD340). Researchers at the NCNPR provided five one-day workshops on botanical dietary supplement authentication techniques to 153 trainees and FDA officials on April 7th, May, 5th, June 23rd, September 8th, and December 5th, 2014. The main training course is held in Memphis, Tennessee so that the trainees can attend a one-day excursion to the NCNPR for a combination of lectures and laboratory courses and training sessions to see what authentication techniques can be implemented for identification of botanicals in BDS. The course covered current techniques utilized to identify botanical materials (microscopy, taxonomy, macroscopy, TLC, HPLC, UPLC, GC, etc.) and was presented by Dr. Khan and colleagues at the NCNPR and included two one and one half hour lab courses on "Analytical Methods, Botanical Authentication, and Nomenclature". It is anticipated that this training program will continue for the foreseeable future and will provide the FDA cGMP botanical dietary supplement inspectors a valuable tool that will assist in their ability to carry out their duties.

Researchers at the NCNPR have also provided their expertise in disseminating the importance of botanicals in dietary supplements. Specifically, Dr. Ikhlas Khan gave several presentations on "Simplification of testing and Analytical Methods-Harmonization of Procedure" in World Spice Congress, Cochin, India, February 16th -19th, 2014; "Multidisciplinary approaches in assuring the quality, safety and efficacy of Traditional Medicines/botanicals" the 9th Vice-Chancellor's Prestigious Research and Innovation Seminar Series, Pretoria, South Africa, March 27, 2014; "Herbal Medicine and Future Development" Shanghai International Conference on Traditional Chinese Medicine and Natural Medicine (S-TCM), Shanghai, China, October 15th, 2014; "Herbal Medicine and Future Development", the 14th International Symposium on Traditional Medicine in Toyama, Toyama, Japan, October 27th -28th, 2014 and several other lectures at national and international meetings. Dr. Yan-Hong Wang gave a lecture on "Current Techniques in Assuring the Quality of Natural Products Used in Dietary Supplements" in 128th AOAC International Annual Meeting & Exposition, September 7-10, 2014, Boca Raton, Florida. Dr. Khan attended the Spices Board India and All India Spice Exporters Forum, organized the World Spice Congress (WSC) in Cochin on February 16th-19th 2014. The highlight of the Congress was the Theme 'Sustainability and Food Safety' which is very relevant and crucial to the current scenario in the food sector. The business sessions planned by the Congress were led by globally renowned industry experts and addressed the topics on sustainable agriculture programs and practices based on real time experiences, infrastructural development, harmonization and simplification of standards etc. Dr. Khan gave a presentation on "Simplification of Testing and Analytical Methods-Harmonization of Procedure" for quality control of several spice products. Dr. Khan received an international award for excellence in Field of Ayurvedic and/or Natural Products by International Association for the Study of Traditional Asian Medicine (IASTAM).

Lastly, the research effort initiated by the establishment of this Center has provided several opportunities to leverage the existing cooperative agreement so as to expand the impact and overall benefit of the existing program. This includes a funded NCCAM/ODS Botanical Research Center grant to explore Botanical Estrogens: Mechanisms, Dose and Target Tissues (PD: Helferich, William G., University of Illinois/PL: Khan, Ikhlas, A., Award number 5 P50 AT006268-04). Under this grant the NCNPR is providing significant quantities

and populations of authenticated samples of licorice - *Glycyrrhiza glabra* Linné var *glabra*, and wild yam - *Dioscorea villosa* L., for the established BRC. In addition to obtaining the outlined authenticated species for this program, we have established a substantial growing and collection program for closely related species for authentication and comparative purposes. Additionally, the NCNPR has obtained funding from the USDA/ARS for a grant entitled "Discovery and Development of Natural Product-Based Insect Management Compounds for Medicinal, Veterinary and Urban Concern", award number 58-6402-1-612. The purpose of this grant is to discover and develop new, safer and more effective insect management products derived from natural phytochemical sources.

Aim 4: Collaborate with FDA scientists in research areas of mutual interest.

Over the past year the NCNPR provided the FDA with scientific information for botanicals of concern as well as investigated several plants that have purported adverse events. Most notably the scientists at Center have undertaken an investigation of "chamomile" which has had reports of contact dermatitis and potential severe cases of allergic reactions in cosmetic formulations. Working closely with scientists in the FDA/CFSAN office of cosmetics and colors, NCNPR initiated an extraction and bioassay guided fractionation of both German and Roman chamomiles utilizing an LLNA screening assay for lead identification. Initial results indicated that there is a potential sensitizer(s) within *Matricaria recutita* L. that could be causing the purported adverse events. Simultaneously, scientists at NCNPR developed two complementary *in chemico* (non-biological, non-animal) methods for identification and classification of chemical compounds as potential skin sensitizers, using either Nuclear Magnetic Resonance (NMR) spectroscopy or High Throughput Spectrophotometric methods. Further investigation including isolation, purification and characterization indicated that the adverse compound as 1,6-dioxaspiro[4,4]non-3-en-2-one (spiro-lactone), a photo-oxidative metabolite of tonghaosu. Several grams of spiro-lactone were synthesized to assess the LLNA potential of this metabolite. Preliminary *in vivo* data indicated that this test article was negative for excessive local irritation (<25% increase in ear thickness) suggesting it is not an irritant. However, based on the stimulation index for this test article, the preliminary data suggest that the test article is a potential dermal sensitizer. Some of the confirmatory tests are still in progress, the exact details and other relevant information will be reported in due-course.

A second project undertaken for CFSAN's office of cosmetics and colors looked at products that include an essential oil known as "Tea tree oil", which is obtained from several species of *Melaleuca* plants. One general research objective for this project is to seek further knowledge on compositional differences between commonly used species of *Melaleuca* plants (*M. alternifolia*, *M. linariifolia*, *M. viridiflora*, *M. leucadendron*, *M. dissitiflora*, etc.) in order to explore ways to address safety concerns for these species. An integrated approach using conventional and chiral GC/MS combined with chemometrics was developed for quality evaluation of terpinen-4-ol-type Australian and commercial tea tree oils.¹⁶

The main safety concern about essential oils from these *Melaleuca* plant species is the potential for adverse effects on the skin, in particular sensitization or allergic contact dermatitis (ACD). It is not fully understood which of the tea tree oil constituents is responsible for ACD. Therefore, several variants of tea tree oils originated from *Melaleuca* species were analyzed using *in-chemico* methods for skin sensation potential. The data obtained from *in-chemico* method was correlated with chemical constituents present in TTO using recently developed analytical GC/MS method. Characterization of plausible sensitizers in TTO is still in progress and the details will be disclosed in the next reporting period.

NCNPR established two in-house preliminary *in-vivo* safety evaluations for botanicals of interest. Priority evaluation was given to botanicals that have displayed an inherent risk based on literature information or reported adverse events. Initial screens focused on two areas of concern. The first mouse model measured botanicals for their potential to induce positive reinforcement or cause aversive properties using a conditioned place preference (CPP) paradigm procedure that is commonly used to evaluate drugs for their "addictive" behavior. Recent work by our group²³ sought to characterize the rewarding properties of *M. speciosa* extract, an alkaloid fraction, and isolated mitragynine in the Conditioned Place Preference (CPP) assay. Mitragynine produced an increase in preference scores similar to the reference article, *d*-amphetamine. Our findings suggest that *M. speciosa* containing significant amounts of mitragynine possesses an abuse liability. Moreover,

due to its perceived μ -opioid receptor agonistic activity, we sought to determine through systemic administration 1) whether activity of *M. speciosa* and mitragynine mirrored opioid activity of morphine and oxycodone and if so, 2) whether these effects are produced via oral routes of administration hotplate assay for antinociception. Based on preliminary studies, mitragynine, but not *M. speciosa* extract, altered nociceptive responses that parallel to oxycodone's antinociceptive properties. Findings of the current research dovetail nicely with earlier studies²³ in rats that reported mitragynine carries a significant abuse potential. Mitragynine's *in vitro* opioid receptor binding profile and *in vivo* activity consistent with known abused opioids is a cause for concern.

As a part of phytochemical investigation, scientists at NCNPR have isolated several known and unknown alkaloids from *S. tortuosum*. Noticeably, these marker components will assist the Center in authentication, identification and development of analytical methods for *S. tortuosum* and its principle alkaloid components. The majority of these findings have not yet been published; however, they will be reported shortly. Similar to *M. speciosa* work, the whole extract, enriched fraction of mesembrine and mesembrine were evaluated for in a number of common, rodent-based assays that model nociception, depression, anxiety, ataxia, and abuse liability.²⁴ Based on our findings, it appears that *S. tortuosum* does not cause preference or aversion in CPP model. Mesembrine appears to have analgesic properties without abuse liabilities or ataxia. The *S. tortuosum* fraction has antidepressant properties but does produce ataxia. The ataxia may limit its usefulness as an antidepressant unless the antidepressant activity is associated with one constituent and the ataxia is associated with a separate constituent. In addition to these behavioral studies, the two major marker compounds, mesembrine and mesembrenone of *S. tortuosum* were assessed for their *in vivo* pharmacokinetic studies and the complete details will be reported soon.

Vinpocetine, a semi-synthetic derivative of vincamine, is currently listed as an ingredient in dozens of dietary supplements sold in the US for the treatment of several CNS related disorders. Despite its wide use, no pharmacokinetic drug interaction studies are reported in the literature. To understand the risk of potentially adverse reactions of vinpocetine with conventional drugs, investigated the effects of vinpocetine on three main regulators of pharmacokinetic drug interactions namely, cytochrome P450s (CYPs), P-glycoprotein (P-gp), and Pregnane X receptor (PXR). Currently, these studies are on-going in our labs and preliminary data suggests that vinpocetine showed a strong inhibition of P-gp (EC_{50} 8 μ M) and a moderate inhibition of recombinant CYP3A4 and 2D6 (IC_{50} 2.8 and 6.5 μ M respectively) with no activity towards 2C9, 2C19 and 1A2 enzymes.

In another study, the inhibitory potential of methanolic extract of *Aegle marmelos* (bael) fruit and its constituents (3 furanocoumarins namely marmelosin, marmesinin, and 8-hydroxypsoralen and 1 alkaloid, aegeline) towards major cytochrome P450 enzymes (CYP3A4, 2D6 and 1A2) using human liver microsomes and recombinant CYPs were studied. Again, these studies are under progress and the initial indication is that, the methanolic extract and marmelosin were found to be competitive and time-dependent inhibitors of CYP3A4 and reversible and non-competitive inhibitors of CYP1A2.

The second *in-vivo* assay was developed to evaluate the potential hepatotoxicity's associated for botanical as measured in a mouse model where the hepatotoxicity is manifested by elevation of aminotransferases and histopathological features of acute hepatocellular necrosis and/or biliary injury. Black Cohosh (BC) (*Cimicifuga racemosa*) has been widely used for the treatment of menopausal symptoms. Sporadic cases of liver toxicity with BC have raised concerns of its safety. Regulatory agencies in different countries have shown concern about the potential association between black cohosh and hepatotoxicity. It is also worth noting, that predisposing conditions that lead to hepatotoxicity in users of BC product is an important factor, but has been frequently overlooked in the clinical reports. Three BC species (*Cimicifuga racemosa*, *Cimicifuga foetida* and *Cimicifuga dahurica*) exhibited no significant effect on liver in healthy mice. However, under health compromised condition (inflammatory state/LPS treatment) the methanol extracts of *C. dahurica* resulted in liver toxicity and mortality. From these preliminary results, we concluded that *C. racemosa* and *C. foetida* spp. of black cohosh did not cause liver toxicity even under inflammatory stimulus. In contrast, *C. dahurica* at high doses (1000 mg/kg and 1200 mg/kg) caused liver toxicity under health compromised condition such as inflammation or fever. Further bioassay guided fractions indicated organic extract of *C. dahurica* showed significant liver injury and mortality in animals that are presensitized with LPS. Even at lower doses (>200

mg/kg), the 100 % mortality within 3 days warrants further phytochemical investigations. It may be necessary to isolate all of the major components and assay them individually to understand the hepatotoxic nature of component derived from *C. dahurica*. The details of phytochemical investigations, *in vivo* data and all other results are currently being evaluated in our labs and will be reported in due course.

Lastly, NCNPR has provided the FDA (CFSAN/OARSA) with scientific information for botanicals of concern as well as investigated several plants that are purported to have hepatotoxicity. Working closely with Dr. Sprando and his colleagues' at OARSA, we identified several sympathomimetic compounds and extracts reported to have hepatotoxic potential. Based on their usage in BDS, five whole methanolic extracts of *Astragalus membranaceus*, *Rauvolfia serpentina*, *Calea zacatechichi*, *Psoralea corylifolia*, *Adhatoda zeylanica*, *Kigelia Africana* were provided to OARSA to estimate the potential toxicity. In addition to these extracts, nine pure compounds were also provided to OARSA. Of nine pure compounds, based on practicality and other factors, four compounds, *N,N*-diethylphenethylamine, *N,N*-dimethylphenethylamine, phenethylamine (PEA), and cocularine were further selected for animal studies. In addition to last year's, 5.0 Kg of cocularine to supply OARSA, this year, 500 grams of *N,N*-diethylphenethylamine hydrochloride was provided. Working closely with DDSP and OARSA, *Bulbine natalensis* (Baker), dendrobine alkaloids, *N,N*-diethyl- β -PEA, *N,N*-dimethyl- β -PEA, *valeriana officinalis*, *morinda citrifolia* and *moringa oleifera* were selected for to *in vitro* study the hepatic potential. We are in process of acquiring the authenticated plant materials and it is anticipated to ship these samples within a few months.

Aim 5: Coordinate scientific workshops and conferences on BDS-related topics of public health relevance to address high priority science and public awareness of emerging problems associated with botanicals.

Due to the focused scheduling of the 13th ICSB (April 15th – 17th, 2014) on regulatory aspects with perspectives from government, manufacturers and trade associations; post market surveillance, risk and safety assessment, quality control and adverse event reporting (AER) for BDS and natural products, contributed presentations or poster submissions were presented in 14th ICSB in conjunction with American Society of Pharmacognosy (ASP) (<http://asp2014.org/>). The 14th ICSB was held on August 2-6th, 2014 in Oxford, MS covering the topics of TCM, Ayurveda, cultivation, collection, post-harvest, processing practices, identification, authentication and quality/safety assessment of botanicals including current clinical evaluations, regulatory aspects and the impact on the global dietary supplement industry. A total of 586 participants from 39 countries representing academia, government and industry and all the proceedings were published in *Planta Medica* (<https://www.thieme-connect.com/products/ejournals/issue/10.1055/s-004-27340>).

The Center hosted the 15th Oxford International Conference on the Science of Botanicals (ICSB) that was held on April 13th – 16th, 2015, at The University of Mississippi, MS. The main theme of the conference was on the progress in the botanical research and development, as well as regulatory and clinical aspects. A special session was announced in the 15th ICSB to detail what has happened, what is being done by industry, retesting programs in response to the New York AG's of issued cease and desist letters to 4 major US retailers alleging that botanical dietary supplements sold under their house brands contained virtually none of the ingredients listed on the label. However, the AG's conclusions were based on evidence provided by DNA-barcoding results. Application of such techniques in BDS were questioned by majority the experts attended the 15th ICSB. Again, all the conference proceedings including oral and poster abstracts for the 15th ICSB were printed in *Planta Medica* 2015, 05, 343-387. (<https://www.thieme-connect.com/products/ejournals/issue/10.1055/s-005-28911>). This conference was co-sponsored by the Shanghai Institute of Materia Medica/CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant and Natural Products Research (GA); the Korean Society of Pharmacognosy and the Vietnam Academy of Science and Technology (VAST) the St Petersburg Institute of Pharmacy; the Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD) and the International Council on Medicinal and Aromatic Plants for Human Welfare (ICMAP) as such included representative delegations of scientists from various organizations in China, India and Europe.

C. Significance:

Plant collection, authentication, voucher specimens, isolation, synthesis of reference compounds and assessment of *in vitro* toxicity provide the basic tools for assessing the safety and quality of botanical products. Significant progress has been made by the NCNPR in each of these areas for several botanicals of interest over the past year. The acquired information, physical samples (plants, extracts, etc.) and phytochemical standards are freely available to researchers at the FDA for evaluation purposes. Publications from this project have significantly contributed to the available methodological literature for the FDA and NCNPR (see references).

D. Plans:

To address the needs of the FDA on safety of BDS, Dr. Ikhlas Khan, Scientific Director for the project, would be in touch with Director, Division of Dietary Supplement Programs and liaison at CFSAN. The center will continue to exchange information on developed methods, reference materials availability, safety evaluations and project direction with CFSAN. Like similar studies of DMAA, continual research effort will also focus on presence/absence of botanical constituents of concern for their potential safety concern due to their abuse potential, undesired adrenergic, dopaminergic receptor activities. For example, several DS products claiming that 1,3-dimethylbutylamine (DMBA), or AMP citrate is a natural constituent of pouchong tea. We obtained several varieties of tea samples through our collaborators at Chinese FDA to establish the authenticity of DMBA from natural sources. Moreover, due to structural similarity with amphetamines, currently, several studies are on-going in our labs for behavioral and potential safety concerns. On April 28, 2015, the FDA issued warning letters to 14 companies regarding a total of 17 products for which the product labeling identifies DMBA as a dietary ingredient. Upon consultation with our program officer, Dr. Welch, several botanicals *mitragyna javonica*, *bulbine natalensis*, *cyanotis vaga*, *fadogia agrestis*, *nettle root*, *afromomum melegueta* were selected for fingerprinting, phytochemical analysis and isolation of marker compounds for safety studies.

Furthering the collaborative research effort we have established with CFSAN's office of cosmetics and colors, additional effort will continue to look at several potential areas of concern. The first being the continued exploration of products that contain tea tree essential oil(s) which can be derived from several species of *Melaleuca* plants. In addition to compositional differences between commonly used species of *Melaleuca* plants (*M. alternifolia*, *M. linariifolia*, *M. viridiflora*, *M. leucadendron*, etc.); the main research objective for this project will be to seek further knowledge on the sensitization potential of these essential oils using recently developed in-house *in chemico* methods to compare and validate the resulting data with other non-animal based methods. At the same time, we will also explore the stability, aging, isolation and identification of possible reactive intermediate(s) in these oils using the recently developed GC-MS analytical method.

Continual research effort will also focus on the two recently developed in-house *in-vivo* screen evaluating botanicals for their potential to induce positive reinforcement or cause aversive properties using the developed CPP paradigm procedure that is commonly used to evaluate drugs for "addictive" behavior and the second assay which evaluates potential hepatotoxicity associated for certain botanicals. Priority evaluation will be given to botanicals that have displayed an inherent risk based on literature information or reported adverse events. For the coming year, we will test DMBA, extracts of *Leonotis leonurus* (wild dagga), *Nelumbo nucifera* (Indian Lotus) for abuse potential. Both models will provide significant insight as to the safety profile for botanicals that are of concern to the public health.

The PI and scientists at NCNPR will continue to work with OARSA by exchanging the scientific information on botanicals of concern with hepatotoxicity potential. For animal studies purpose, on bulk scale, three other compounds, *N,N*-dimethylphenethylamine, phenethylamine, dendrobine would be provided to OARSA. By working closely with OARSA, the Center will continue to provide significant insight into the authentication, validation, analytical and safety profile for BDS that are of hepatotoxic concern to public health.

Lastly, expansion of the existing repository of reference plant samples for the genera noted throughout this report will be a continuing priority for this project. New botanicals will be selected based on input received from the FDA Program Officer, collaborators and liaison for further studies and to evaluate their safety and quality. A sixteenth Oxford International Conference on the Science of Botanicals (ICSB) is proposed to be held on April

11th – 14th, 2016, Oxford, MS. The conference will be co-sponsored by the Shanghai Institute of Materia Medica/CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the Ministry of Indigenous Medicine, Sri Lanka; the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant and Natural Products Research (GA); the Korean Society of Pharmacognosy and the Vietnam Academy of Science and Technology (VAST) the St Petersburg Institute of Pharmacy; the Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD) and the International Council on Medicinal and Aromatic Plants for Human Welfare (ICMAP). This conference will cover the topics of TCM, Ayurveda, cultivation, collection, post-harvest, processing practices, identification, authentication and quality/safety assessment of botanicals including current clinical evaluations, regulatory aspects and the impact on the global dietary supplement industry. Again the proceedings from this conference are expected to be published in *Planta Medica*.

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ALL PERSONNEL REPORTGRANT NUMBER
U01FD004246

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Always list the PD/PI(s). In addition, list all other personnel who participated in the project during the current budget period for at least one person month or more, regardless of the source of compensation (a person month equals approximately 160 hours or 8.3% of annualized effort). Use the following abbreviated categories for describing Role on Project:

- PD/PI*
- Co-Investigator
- Faculty
- Postdoctoral (scholar, fellow, or other postdoctoral position)*
- Technician
- Staff Scientist (doctoral level)
- Statistician
- Graduate Student (research assistant)
- Non-student Research Assistant
- Undergraduate Student
- High School Student
- Consultant
- Other (please specify)

If personnel are supported by a Reentry or Diversity Supplement please indicate such after the Role on Project, using the following abbreviations: RS - Reentry Supplement; DS - Diversity Supplement.

*Commons ID required for any personnel holding this Role on Project and for all individuals supported by a Reentry or Diversity Supplement. The Commons ID will be required in the future for all individuals with a graduate student, or undergraduate role. The Commons ID is strongly encouraged, but not required, for all other Project Personnel.

Use Cal (calendar), Acad, or Summer to enter months devoted to project.

Commons ID*	Name	Degree(s)	SSN (last 4 digits)	Role on Project	DoB (MM /YY)	Cal	Acad	Summer
IKHLAS	Ikhlas Khan	Ph.D.	(b) (6)	PI	(b) (6)	6.0		
LARRYWA LKER2004	Larry Walker	Ph.D.		Co-PI		1.2		
SKHAN	Shabana Khan	Ph.D.		Staff Scientist		3.0		
BAVULA	Bharathi Avula	Ph.D.		Staff Scientist		9.6		
YAN HONG	Yan Hong Wang	Ph.D.		Staff Scientist		12		
	Natasha Tehen	Ph.D.		Staff Scientist		12		
ALI	Zulfiqar Ali	Ph.D.		Staff Scientist		11.4		
CHITTIBOY INA	Amar Chittiboyina	Ph.D.		Staff Scientist		7.2		
	Gouyi Ma	Ph.D.		Staff Scientist		12		
	Ahmad Osman	Ph.D.		Staff Scientist		12		
JPZHAO	Jianping Zhao	Ph.D.		Staff Scientist		6.36		
VRAMAN	Vijayasankar Raman	Ph.D.		Postdoc		12		
	Mei Wang	Ph.D.		Postdoc		11.4		

ALL PERSONNEL REPORT

GRANT NUMBER

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- Co-Investigator
- Faculty
- Postdoctoral (scholar, fellow, or other postdoctoral position)*
- Technician
- Staff Scientist (doctoral level)
- Statistician
- Graduate Student (research assistant)
- Non-student Research Assistant
- Undergraduate Student
- High School Student
- Consultant
- Other (please specify)

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Use Cal (calendar), Acad, or Summer to enter months devoted to project.

Commons ID*	Name	Degree(s)	SSN (last 4 digits)	Role on Project	DoB (MM /YY)	Cal	Acad	Summer
	Christina Avonto	Ph.D.	(b) (6)	Postdoc	(b) (6)	12		
	Sanaz Salehi	Ph.D.		Postdoc		12		
	Gray Dale	MA		Rsch Asst		1.2		
	Zhihao Zhang	Ph.D.		Postdoc		12		
	Pradeep Lasonkar	Ph.D.		Postdoc		12		
	Iffat Parveen	Ph.D.		Postdoc		12		
	Jessica Carpenter	M.A.		Rsch Asst		12		
	Saqlain Haider	Ph.D.		Postdoc		12		
	Satyanarayanaraju Sagi	Ph.D.		Postdoc		12		
	Ji-Yeong Bae	Ph.D.		Postdoc		12		
	Lal Jayaratna	MSc		Rsch Asst	(b) (6)	12		
	Steven Hopper	BFA		Technician		12		
	Jennifer Taylor			Rsch Asst		12		

Program Director/Principal Investigator (Last, First, Middle): Khan Ikhlas A

PROGRESS REPORT SUMMARY	GRANT NUMBER 1U01FD004246	
	PERIOD COVERED BY THIS REPORT	
	FROM 09/15/2011	THROUGH 08/31/2012
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Khan, Ikhlas, A		
APPLICANT ORGANIZATION The University of Mississippi		
TITLE OF PROJECT (Repeat title shown in Item 1 on first page) Science Based Authentication of Dietary Supplements		
A. Human Subjects (Complete Item 6 on the Face Page)		
Involvement of Human Subjects	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change
B. Vertebrate Animals (Complete Item 7 on the Face Page)		
Use of Vertebrate Animals	<input type="checkbox"/> No Change Since Previous Submission	<input checked="" type="checkbox"/> Change
C. Select Agent Research	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change
D. Multiple PD/PI Leadership Plan	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change
E. Human Embryonic Stem Cell Line(s) Used	<input checked="" type="checkbox"/> No Change Since Previous Submission	<input type="checkbox"/> Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

None

Progress Report Summary

A. Specific Aims

Under the provisions of the DSHEA, the FDA has primary responsibility for ensuring that appropriate regulatory actions are taken against marketed products that present significant health risks or bear false or misleading label claims. Foundational to the evaluation of such risks and claims is an adequate scientific base for decision-making. For botanical dietary supplements, development of such a science base is especially problematic because of several unique features, including the complexity of the constituents, variability of sourcing, lack of availability of reference materials, lack of manufacturing controls and rapidly expanding use in the marketplace. In 2010 the FDA fully implemented the current good manufacturing practices (cGMP) for the botanical dietary supplement industry placing the onus of "botanical identity and authenticity" on the manufacturers of botanical dietary supplements. However, these cGMP's have in many ways increased the complexity as to what constitutes a "scientifically valid method" for the determination of the identity and authenticity of botanical materials. To address these complex issues surrounding botanical identity, authenticity, and safety and in continuation of our cooperative agreement with the FDA, the NCNPR has established the following specific aims to aid in the research needs of the FDA:

1. Assist in the identification and development of a list of BDS and botanical ingredients, based on safety concerns, trends, and knowledge of botanicals being marketed in the U.S., to prioritize for further research.
2. Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to FDA, for evaluation of their safety.
3. Exchange technical and scientific information, analytical methods, and reference material with FDA scientists and other stakeholders.
4. Collaborate with FDA scientists in research areas of mutual interest.
5. Coordinate scientific workshops and conferences on BDS-related topics of public health relevance to address high priority science and research needs.

This renewal request expands the research under the current agreement on BDS to include potential safety issues, and extends the effort to include additional emerging problems associated with botanicals.

B. Studies and Results:

Aim 1: Identify botanical dietary supplements (BDS) of priority concern to FDA from a public safety perspective and determine research needs.

Since the inception of the cooperative agreement between the NCNPR and the FDA in 2001, there have been numerous occasions for interactions between the two organizations. These have included (yearly) formal site visits by the FDA program officers, interactions during the annual International Conference on the Science of Botanicals (ICSB) and several conference calls and email exchanges. As always, the researchers at the NCNPR are receptive to areas of study that are deemed necessary by the FDA for this project. As such special attention has been given to botanical species that have raised concern including; *Acacia rigidula*, *Bauhinia purpurea*, *Cirsium oligophyllum*, *Barosma betulina*, *Kigelia africana*, *Justicia adhatoda*, *Aloe barbadensis*, *Achillea millefolium*, *Arnica Montana*, and *Mucuna pruriens*.

Aim 2: Acquire, validate, and characterize authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to FDA, for evaluation of their safety.

The availability of authenticated reference materials for botanicals is an essential first step in the development of analytical methodologies for the identification and quantitative analysis of the product(s). The NCNPR has been very successful in placing formal agreements with several international academic and governmental institutions including Pakistan, India (FRLHT), South Africa, Central/South America, China with the development of the Sino-US TCM Research Center and the established Center for Research in Indian Systems of Medicine (CRISM - www.CRISM.net) with the departments of AYUSH and CSIR in India. The NCNPR has also established a Memorandum of Understanding (MOU) with the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Brazil and the Natural Products Utilization Research Unit (NPURU), USDA located in Oxford Mississippi. Lastly the NCNPR is cultivating a relationship with the Chinese FDA in order to

obtain relevant authenticated Traditional Chinese Medicines (TCM's) and pertinent purified constituents for authentication purposes.

From these and other collaborative relationships, the NCNPR has been able to acquire a total of 5300 plant samples and herbal extracts, representing over 1500 species. Additionally, over the past year, researchers at Harvard Medical School donated over 300 authenticated bulk samples from a collaborative collection that was acquired for a project examining TCM materials. These samples were included into the NCNPR-FDA repository in addition to our regular annual collections of authenticated samples. Lastly, we are continually acquiring commercial samples as well for authentication purposes in addition to assuring that the developed identity techniques for this project are applicable for finished products in various formularies. Taking all these sample types into account there are over 12,000 specimens within the NCNPR-FDA repository. All the plants and samples collected for this program have been assigned a inventory number and are cataloged in the NCNPR repository and voucher specimens of authentic samples are also maintained at the Thomas M. Pullen Herbarium in the Department of Biology at the University of Mississippi (MISSA).

In addition to this the NCNPR has a newly renovated Medicinal Plant Garden that maintains more than 1200 species for selected growing (field, greenhouse and shade houses). The new facilities consist of two main buildings (4,362 sf. and 4,290 sf.) and four additional support buildings and structures sitting on approximately 5.25 acres of land. The Medicinal Plant Garden has developed significant collaborations with seed banks from 40 institutes from around the world in order to exchange medicinally important germplasms. In addition, the garden personnel are preparing herbarium voucher specimens from the living collection and have started collecting samples for a DNA tissue bank. Over the past year the garden provided 288 authentic reference samples from this living collection for the Centers research efforts. The Medicinal Plant Garden is also registered under the Botanic Gardens Conservation International and is a member of the American Association of Botanical Gardens and Arboreta and International Plant Exchange Network (IPEN).

Isolation of reference compounds: The isolation of standard compounds is required to develop analytical methods, quantify individual compounds in extracts and to establish analytical fingerprints in support of quality and safety studies. Scientists at the NCNPR have isolated a number of compounds from, *Casearia sylvestris*, *Scutellaria lateriflora*, *Curcuma longa*, *Matricaria recutita* L., *Anthemis nobilis*, *Rhodiola spp.*, *Hoodia gordonii*, *Actaea racemosa*, *Lonicera Japonica Thunb.*, *Caulophyllum thalictroides*, and *Sutherlandia frutescens*. It is through these continued efforts that the NCNPR scientist have isolated dozens of novel compounds as well as known analogs for analytical finger printing development for authentication purposes as well as safety analysis.

Analytical method development and metabolomic profiling: In the past year we have focused on developing several analytical methods for the determination of authenticity BDS's of interest. These studies include the development of UPLC (UV, ELS and MS), standard HPLC/HPTLC analytical methods as well as using proton NMR for metabolomic profiling for common botanicals including *Morus spp.*, *Lepidium meyenii* (Maca), *Rhodiola spp.*, *Lonicera Japonica Thunb.*, *Lancea tibetica*, *Curcuma spp.*, *Scutellaria lateriflora*, *Pausinystalia johimbe* (yohimbe), *Ginkgo biloba*, *Sutherlandia frutescens*, and *Hoodia gordonii*.

Aim 3: Exchange technical and scientific information, analytical methods, and reference material with FDA scientists and other stakeholders.

Dr. Ikhlas Khan, Scientific Director for the project, has been in touch regularly with Dr. Daniel Fabricant (CFSAN, Director, Division of Dietary Supplement Programs), Dr. Elizabeth Calvey (CFSAN liaison) and Dr. Diego Rua (CFSAN) to exchange information on developed methods, reference materials availability, safety evaluations and project direction. These meetings and calls are designed to provide these individuals with an update of the NCNPR's overall program and to gain insight as to how this center can better address the needs of the FDA.

Additionally, the NCNPR FDA-COE has also continued to assist the FDA's Office of Regulatory Affairs (ORA) in training inspectors for cGMP compliance for BDS's (FD340). Researchers at the NCNPR provided a one-day workshop on botanical dietary supplement authentication techniques to over 50 trainees and FDA officials

on May 17th 2012. The main training course is held in Memphis, Tennessee so that the trainees can have a one-day excursion to the NCNPR for a combination of lectures and laboratory tours and training sessions to see what authentication techniques can be implemented for BDS's. The course covered current techniques utilized to identify botanical materials (Microscopy, Taxonomy, Macroscopy, TLC, HPLC, UPLC, GC, CE, etc) and was presented by Dr. Khan and colleagues at the NCNPR and included three one hour lab courses on Analytical Methods, Botanical Authentication, and Nomenclature. It is anticipated that this training program will continue for the foreseeable future and will provide the FDA cGMP botanical dietary supplement inspectors a valuable tool that will assist in their ability to carry out their duties.

Additionally, researchers at the NCNPR have provided their expertise in other training offered by the FDA/ORA/DHRD. The course was an advanced level course for analysts who are performing regulatory sample analysis using mass spectrometry techniques for identification and authentication (LB 403). Specifically, Dr. Yan-Hong Wang provided a lecture to several FDA trainees on March 1st, 2012 covering the topic of how mass spectroscopy can be utilized for the authentication of botanicals. A second course is scheduled for August 26th – 31st, 2012 and we anticipate that we will continue to offer our expertise to the FDA on topics similar to this throughout the scope of the grant. The course will again focus on both the theoretical aspects and practical applications of LC-MS and GC-MS in regulatory analyses through lectures and hands-on laboratory sessions where Dr. Wang will again offer a lecture on MS techniques and how they can be utilized for BDS's.

Lastly, the research effort initiated by the establishment of this center has provided several opportunities to leverage the existing cooperative agreement so as to expand the impact and overall benefit of the existing program. This includes a recently funded NCCAM/ODS Center grant to explore Botanical Estrogens: Mechanisms, Dose and Target Tissues (PD: Helferich, William G., University of Illinois/PL: Khan, Ikhlas, A., Award number 5 P50 AT006268-02). Under this grant the NCNPR is providing significant quantities and populations of authenticated samples of Licorice - *Glycyrrhiza glabra* Linné var *glabra*, Wild Yam - *Dioscorea villosa* L., and Dong Quai - *Angelica sinensis* (Oliv.) Diels for the established BRC. In addition to obtaining the outlined authenticated species for this program we have established a substantial growing and collection program for closely related species for authentication and comparative purposes. Lastly, the NCNPR has obtained funding from the USDA/ARS for a grant entitled Discovery and Development of Natural Product-Based Insect Management Compounds for Medicinal, Veterinary and Urban Concern, award number 58-6402-7-228. The purpose of this grant is to discover and develop new, safer and more effective insect management products derived from natural sources.

Aim 4: Collaborate with FDA scientists in research areas of mutual interest.

Over the past year the NCNPR has provided the FDA with scientific information for botanicals of concern as well as investigated several plants that have purported adverse events. Most notably we have undertaken an investigation of "chamomile" which has had reports of contact dermatitis and potential severe cases of allergic reactions in cosmetic formulations. The initial question, as with all botanicals, is what is meant when discussing "chamomile" such as which species or variety was used, what plant part, what processing method was used, etc. As it turns out there are two main species of "chamomile" utilized for commerce within the United States German chamomile, *Matricaria recutita* L. and Roman chamomile, *Anthemis nobilis*. Typically the flowering tops are used for most cosmetic formulations and these are either added as powdered material or an extract (ethanolic, supercritical or steam distilled). Working closely with scientist in the FDA/CFSAN office of cosmetics and colors, we initiated an extraction and bioassay guided fractionation of both German and Roman chamomile utilizing an LLNA screening assay for lead identification. Initial results are indicated that there is a potential sensitizer within *Matricaria recutita* L. that could be causing the purported adverse events. Further investigation including isolation, purification and bioassay evaluations will be undertaken in the next project period to identify the constituent(s) that contribute to the observed sensitization.

Aim 5: Coordinate scientific workshops and conferences on BDS topics of public health relevance to address high priority science and research needs.

The Center hosted the 11th Oxford International Conference on the Science of Botanicals (ICSB) that was held on April 16th – 19th 2012, at The University of Mississippi. This conference was co-sponsored by the Shanghai

Institute of Materia Medica/CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the Ministry of Indigenous Medicine, Sri Lanka; the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant and Natural Products Research (GA); the Korean Society of Pharmacognosy and the Vietnam Academy of Science and Technology (VAST) and included representative delegations of scientists from various organizations in China, India and Europe. In addition to this there were representatives from several well known international organizations as both attendees and presenters to total over 220 participants at this conference. The conference also included a commemorative session honoring Norman Farnsworth and his contributions to the field of natural products and botanical research as well as a specific session dedicated to highlight the scientific efforts of the established ODS/NCCAM Botanical Research Centers. The abstracts for this conference have been published in *Planta Medica*, 2012, 78(5), 497-552.

C. Significance:

Plant collection, authentication, voucher specimens, isolation of reference compounds and method development provide the basic tools for assessing the safety and quality of botanical products. Significant progress has been made by the NCNPR in each of these areas for several botanicals of interest over the past year. The acquired information is freely available to researchers at the FDA as well as physical samples (plants, extracts, etc) and phytochemical standards for evaluation purposes. Publications from this project have significantly contributed to the available methodological literature for the FDA and NCNPR (see references).

D. Plans:

Furthering the collaborative research effort we have established with CFSAN's office of cosmetics and colors, additional effort will be allotted to look at two potential areas of concern. The first being the investigation of cosmetic products that contain β -arbutin. β -arbutin is a natural product that can be extracted from plants but can also be made synthetically. Cosmetics manufacturers are purportedly adding β -arbutin to products as a skin-whitening agent. However the EU Scientific Committee on Consumer Products (SCCP) determined in 2008 that β -arbutin in cosmetic products should be considered unsafe. Additionally in 2006, the FDA issued a notice of proposed rulemaking to establish that all skin bleaching products, whether marketed on a prescription or OTC basis, are drugs requiring an approved new drug application (NDA) for continued marketing. However the corresponding final rule has not been issued. The main safety concern for β -arbutin is that it can be a source for hydroquinone, which has displayed carcinogenicity in animals and poses a potential carcinogenic risk in humans. In addition, hydroquinone has been shown to cause disfiguring effects (ochronosis) after topical use at concentrations as low as 1 to 2-percent. Since β -arbutin can be extracted from plants, one general research objective would be to seek further knowledge regarding which plants are best sources of β -arbutin such as *Salvia officinalis*. There is also speculation that some manufacturers are utilizing synthetic arbutin within their products in place of natural sources. Both of these issues can be investigated by developing an analytical method to differentiate between α -arbutin (synthetic) and β -arbutin (natural). After establishing the method and validating it for plant sources that are known to contain arbutin, then we can utilize this method to analyze several (~30) botanical products that claim to contain natural β -arbutin from plant sources to see what levels of α and β arbutin each product contains. This project will aid FDA in assessing whether restricting the concentration of use of β -arbutin and/or other cosmetic ingredient sources of hydroquinone is needed in order to prevent skin adverse effects such as ochronosis and promote public health.

The second project we will undertake for CFSAN's office of cosmetics and colors will look at products that include an essential oil known as "Tea tree oil", which is obtained from several species of *Melaleuca* plants. One general research objective for this project will be to seek further knowledge on compositional differences between commonly used species of *Melaleuca* plants (*Melaleuca alternifolia*, *Melaleuca linariifolia*, *Melaleuca viridiflora*, *Melaleuca leucadendron*, *Melaleuca dissitiflora*, etc.) in order to explore ways to address safety concerns for these species. The main safety concern about essential oils from these plant species is the potential for adverse effects on skin, in particular skin sensitization or allergic contact dermatitis (ACD). It is not fully understood which of the Tea tree oil constituents is responsible for ACD. Therefore, the potential ACD active(s) in Tea tree oil will be identified and its corresponding no-observable adverse effect level (NOAEL) will be empirically determined by *in vitro* cell-based assays. This will aid FDA in assessing whether restricting use of Tea tree oil in cosmetics is needed in order to prevent skin adverse effects and promote public health.

Additional research areas have also been identified after extensive discussions with our program officer. The FDA has a need to establish preliminary safety evaluation for many botanicals of interest, therefore the COE will develop two preliminary *in-vivo* safety evaluation screens for this purpose. Priority evaluation will be given to botanicals that have displayed an inherent risk based on literature information or reported adverse events. Initial screens will focus on two areas of concern. The first assay will evaluate potential hepatotoxicity's associated for botanical as measured in a mouse model where the hepatotoxicity is manifested by elevation of aminotransferases and histopathological features of acute hepatocellular necrosis and/or biliary injury. The second mouse model will measure botanicals for their potential to induce positive reinforcement or cause aversive properties using a conditioned place preference paradigm procedure that is commonly used to evaluate drugs. Both models will provide significant insight as to the safety profile for botanicals that are of concern to the public health.

Lastly, expansion of the existing repository of reference plant samples for the genera noted throughout this report will be a continuing priority for this project. New botanicals will be selected based on input received from the FDA program officer, collaborators and liaison for further studies and to evaluate their safety and quality. A twelfth conference is being organized entitled Oxford International Conference on the Science of Botanicals (ICSB) to be held on April 15th – 18th, 2013 at The University of Mississippi. This conference will be co-sponsored by the Shanghai Institute of Materia Medica/CAS, China; the Council of Scientific and Industrial Research (CSIR - India); the Ministry of Indigenous Medicine, Sri Lanka; the American Society of Pharmacognosy (ASP); the Society for Medicinal Plant and Natural Products Research (GA); the Korean Society of Pharmacognosy and the Vietnam Academy of Science and Technology (VAST). This conference will cover the topics of TCM, Ayurveda, cultivation, collection, post-harvest, processing practices, identification, authentication and quality/safety assessment of botanicals including current clinical evaluations, regulatory aspects and the impact on the global dietary supplement industry. Again the proceedings from this conference are to be published in *Planta Medica* in 2013.

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